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International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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Dr. D. J. HISSINK, IN MEMORIAM

On January 17th, 1956, Dr. D. J. Hissink died at his home in Haren, near Groningen, at the age of 81 years. A long life, rich in accomplishment was ended; an outstanding scientist, a strong personality, passed away; in him Holland lost one of her best sons, and soil scientists in many countries, a revered colleague, friend and comrade. The results of his labour live on.

David Jacobus Hissink was born in Kampen, in the Netherlands, on October 22nd, 1874. He studied the Natural Sciences at the University of Amsterdam and received his doctor's degree from that institution. In 1899 he left for the colonies where he was appointed agricultural chemist to the Botanical Gardens at Buitenzorg, Java. Four years later he returned to his native land as chemist for the Royal Dutch Agricultural Research Station at Goes of which he soon became director. In 1916 he was made director of the newly established Soil Science Institute at Groningen, and there he remained for 23 years until he resigned in 1939.

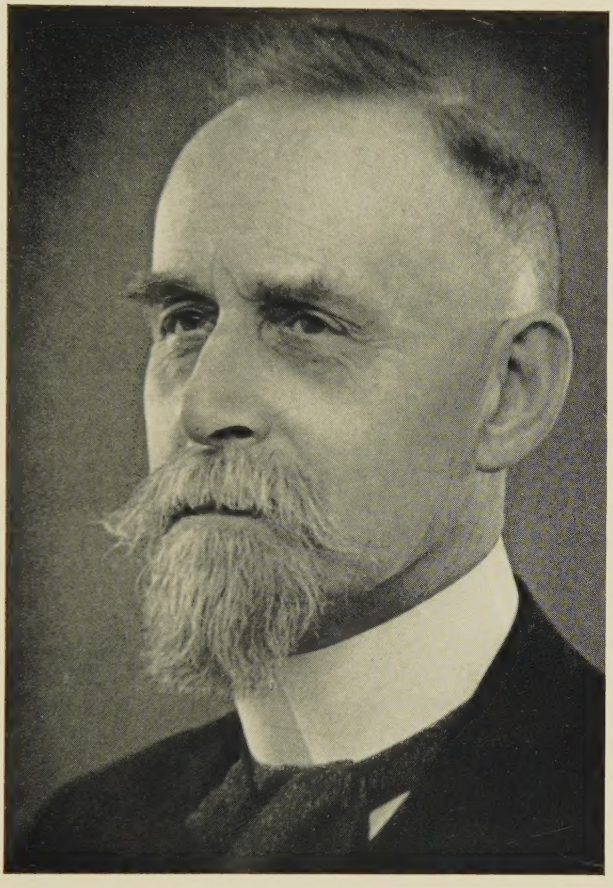
Hissink's work, of deep and lasting influence on pedology, centered on exchangeable bases and their effect on the structure and permeability of soils, a problem of outstanding importance in the reclamation of areas recently covered by sea water. Hissink was the first to demonstrate the rôle of sodium ions in those changes of structure which always appear at a certain stage during the leaching of sea-flooded soils. It was Hissink who devised quantitative methods to determine the total and relative quantities of adsorbed exchangeable bases in such soils. He defined and used the term "degree of base saturation" and demonstrated the close connection between this figure and the pH value of a soil. His work, therefore, was also of primary importance in the study of soil acidity and of the entire liming problem. Hissink's T, S and V values have gained international recognition, and are quoted today in all the important pedological text and reference books.

Many of Hissink's investigations were connected with the Zuyder Zee project, a national reclamation enterprise of gigantic dimensions, unequalled in history and renowned throughout the civilized world. It is largely to Hissink's credit that this great project has thus far developed according to plan with none of the deceptions and failures so often associated with similar projects on a much smaller scale.

Hissink and his collaborators investigated and charted the future soils of the Wieringermeer and Noordoostpolder, when these large areas were still covered by the sea. They planned the canal system and drainage of new land, studied its de-salting by chemical analyses, and proved, at the same time, the influence of sodium on drainage. Later, when the areas had been cultivated, they studied the gradual change of sea bottom into soil under the influence of climate. All these carefully planned investigations were pioneer in nature and shed new light on many soil problems of great theoretical and practical importance. No wonder, therefore, that numerous soil scientists, experts in land reclamation and practical farmers from many countries, flocked to Holland to see and learn from the astonishing results which, as a life-bringing spring, emerged and spread from Hissink's institute at Groningen.

No small part of Hissink's talents and great capacity for work were placed at the service of the International Society of Soil Science He was one of the founders of this society in Rome, in 1924, when he was elected president and secretary general. The presidency changed every five years but Hissink continued as secretary general and *primus motor* of the society for many years. With great skill and never failing interest he developed and directed the manifold functions of the I.S.S. He established and maintained contacts with foreign sections and various commissions, prepared conferences, commission meetings and congresses so that his institute at Groningen became an international centre for soil science. During the period, 1924–40, three large scale congresses and ten commission meetings were held under his auspices in nearly as many different countries; Hissink himself took part in most of them and often led the discussions.

During the second world war all international ties and also those of soil science were broken, but immediately after the armistice in 1945, Dr. Hissink re-established connections. Partly as a personal



D. J. HISSINK 1874–1956

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tribute to him, the 4th International Congress of Soil Science was held in Amsterdam in 1950. On this occasion his great services and unselfish work for the society were emphasized and highly praised and he was made a honorary member. From that date he took no active part in the society.

His last years were spent quietly in "Huize Avondlicht", Haren, near Groningen, but not in idleness. His keen interest in the great achievement of his life, the reclamation and cultivation of the Dutch polder and marsh areas, was unabated. He took an active part, as adviser to the Dutch government, in land reclamation by gypsum treatment following the flood catastrophes of 1953, and he continued his studies on the humus content and nitrogen cycle of the young Zuyder Zee soils.

The unique character of D. J. Hissink's work was recognized and highly esteemed in wide circles. The most exclusive decorations of his own country were bestowed upon him for distinguished service, and he was made a honorary member of numerous scientific societies in foreign countries. Any one who has had the privilege of knowing him as a friend and colleague will always remember his highmindedness and distinction. In scientific discussions he expressed his opinions in a concise manner, often with humour, and he defended them stubbornly with well-chosen, though not always gentle words. Behind a sometimes brusque demeanour was a sensitive nature and a warm heart.

Hissink was an intellectual aristocrat and a pure idealist. In his opinion the primary duty of man was to conquer the earth solely by improved cultivation of the soil for the benefit of mankind. His life and work confirmed his ideals.

Now he is dead. We can never again walk with him over the flat, fertile land which he had won from the sea and loved so deeply; we can no longer hear his voice in stimulating discussion, but we will carry his memory as an inspiration in our hearts.

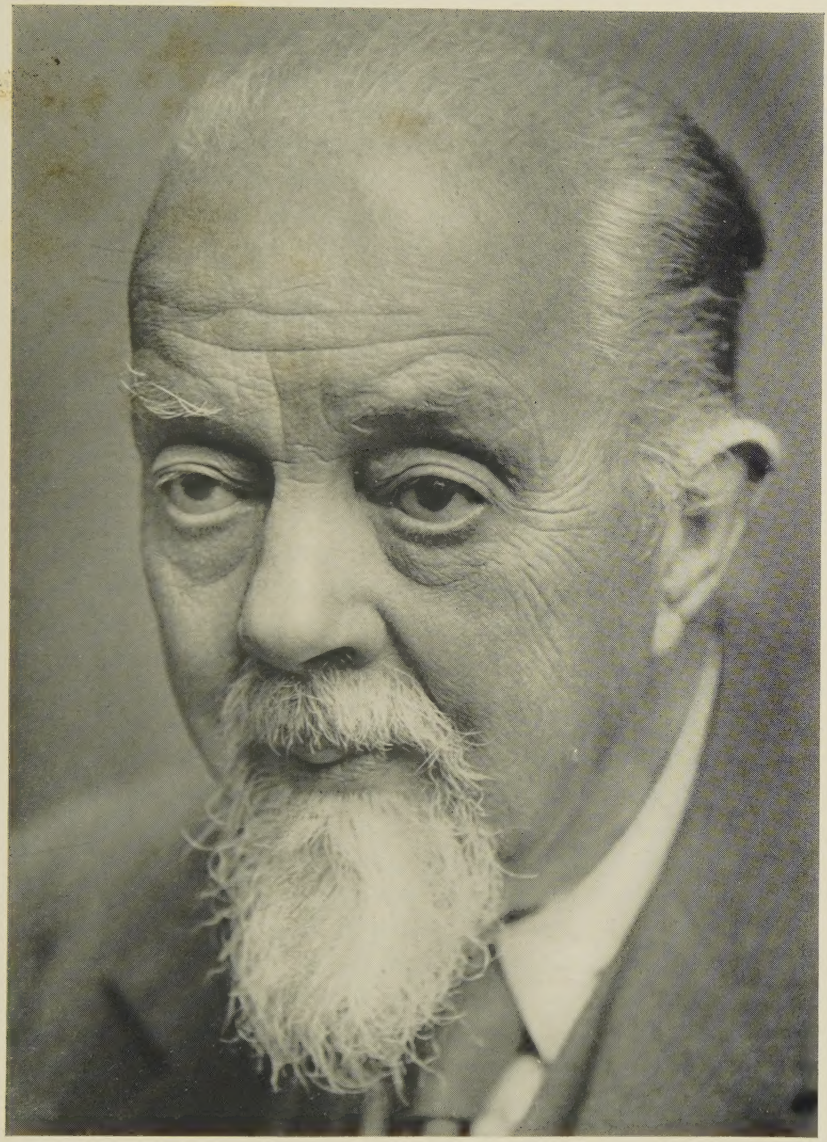
Copenhagen S. Tovborg Jensen

E. A. MITSCHERLICH ZUM GEDENKEN

Der Tod von Eilhard Alfred Mitscherlich am 3. Februar 1956 traf, trotz seines hohen Alters, für die Welt der Landwirtschaftswissenschaft unerwartet ein. Der rege Geist des großen Forschers hatte bis zur letzten Zeit mit unverminderter Kraft und jugendlichem Schwung wissenschaftlich-schöpferisch gewirkt und nichts von der körperlichen Krankheit der letzten Jahre merken lassen.

Geboren am 29. August 1874 in Berlin als Sohn eines Chirurgie-Professors und Enkelsohn des berühmten Chemikers Eilhard Mitscherlich, gewann er bereits als Jüngling in den Ferien einen innigen Kontakt mit der praktischen Landwirtschaft auf den Gütern Langenöls und Kutschlau seiner Großeltern mütterlicherseits. Hier hatte er Gelegenheit zu seinen ersten Beobachtungen als Naturforscher; der Schüler machte Schmetterlingssammlungen und der Student der Landwirtschaft und der Doktorand benutzte die Böden dieser Güter für die ersten wissenschaftlichen Untersuchungen, wie z.B. zu den Bestimmungen der Benetzungswärme und der Hygroskopizität.

Sein landwirtschaftliches Studium an der Universität Kiel ergänzte Mitscherlich in München und Berlin mit weiteren Arbeiten in chemischen und physikalischen Laboratorien und durch Teilnahme an Vorlesungen über höhere Mathematik. Als er wissenschaftlicher Assistent bei seinem großen Lehrer und Vorbild Hermann Rodewald in Kiel wurde, verfügte der junge Wissenschaftler über ausreichend breite Grundlagen zur vielseitigen Erforschung eines verhältnismäßig jungfräulichen Gebietes, wie es die Landwirtschaft damals darstellte. Diese Grundlagen wirkten sich bereits bei seinen ersten Arbeiten aus, indem er durch die Bestimmung der *Benetzungswärme* und der *Hygroskopizität* eine neue physikalische Betrachtungsweise des Bodens welche über diejenige von Wollny hinausragte, schuf. Die in



EILHARD ALFRED MITSCHERLICH 1874–1956

den Jahren 1898 bis 1901 ausgearbeitete Methode zur Bestimmung der Hygroskopizität wurde auch international anerkannt und von der ganzen Fachwelt übernommen. Gleichzeitig begann er der Verarbeitung von landwirtschaftlichen Versuchsergebnissen eine objektive Grundlage zu schaffen, indem er als einer der Ersten sich dabei für die Anwendung der Fehlerrechnung einsetzte.

Obwohl noch sehr jung, konnte er 1905 die erste Auflage seines Buches: „Bodenkunde für Land- und Forstwirte" herausgeben. Auf sehr viel eigen erarbeitetem Material fußend, brachte das Buch eine neuartige Auffassung der Problemstellung und der Untersuchungsmethodik des Bodens als Träger unserer Kulturpflanzen. Mitscherlich definierte es im Vorwort seines Buches wie folgt:

„.... denn es ist für unsere Kulturpflanzen ganz gleichgültig, wie der Boden, auf dem sie wachsen, einst geologisch entstand; das Gedeihen der Pflanzen wird sich stets danach richten müssen, wie der Boden momentan physikalisch und chemisch beschaffen ist".

Den Boden selbst definiert er:

„Boden ist ein Gemenge von mehr oder minder kleinen festen Teilchen, Wasser und Luft, welches, versehen mit den erforderlichen Pflanzennährstoffen, als Träger einer Vegetation dienen kann".

Das Buch fand allgemeine Anerkennung und brachte ihm bereits im Jahre 1906 die Berufung zum Professor für Pflanzenbau an der Albertus-Universität zu Königsberg.

Sein Sinn für exakte Forschung, unterstützt durch ausreichende physikalisch-chemische und mathematische Kenntnisse, führte ihn sehr bald dazu, Experimente anzustellen, welche die Wirkung der Faktoren, die den Pflanzenertrag bestimmen, erkennen lassen konnten. Dafür stellte er die Versuche in der Weise an, daß bei ausreichenden, gleichgehaltenen Mengen aller übrigen Faktoren nur der zu untersuchende variiert wurde.

Die ersten Ergebnisse dieser seiner Arbeiten veröffentlichte Mitscherlich im Jahre 1909 in den Landwirtschaftlichen Jahrbüchern (Bd. XXXVIII) unter dem Titel: „Das Gesetz des Minimums und das Gesetz des abnehmenden Bodenertrages". Die nun *quantitativ* erfaßten Beziehungen zwischen Nährstoffmenge und Ertrag ließen erkennen, daß die dabei vorhandene Abhängigkeit

eine Exponentialfunktion darstellen kann. Er drückte sie in folgender Differentialgleichung aus:

$$\frac{dy}{dx} = (A - y).k$$

welche, im Gegensatz zu der bis dahin in Anlehnung an das Liebig'sche Gesetz vertretenen Meinung der linearen Funktion, eine stete Abnahme der Wirkung zusätzlicher Mengen des Faktors x zeigt. Die anschließenden, zahlreichen Arbeiten führten ihn 1913 zur Formulierung des „Gesetzes der physiologischen Beziehungen", welches er später (1920) mit B. Baule in „Das Wirkungsgesetz der Wachstumsfaktoren" umbenannte.

Zur gleichen Zeit konnte er auch die Abhängigkeit des Wachstums von der Zeit quantitativ erfassen und formulierte im Jahre 1919 „das Gesetz des Pflanzenwachstums".

Mit diesen Arbeiten betonte Mitscherlich immer mehr die Heranziehung der Pflanze und des Pflanzenertrages als Indikator zum Studium der Wachstumsfaktoren, bzw. des Bodens als Ertragsfaktor und schuf somit die „pflanzenphysiologische Bodenkunde". Dadurch gab er den, von ihm erarbeiteten, *theoretischen* Erkenntnissen eine große, *praktische* Bedeutung.

Seine Anschauungen und Arbeiten fanden in der ganzen Welt großen Widerhall und dienten als Anregung für unzählige weitere Forschungen, aber sie wurden auch gleichzeitig unter den verschiedensten Bedingungen in ihrer Gültigkeit überprüft. Die praktische Anwendung der Beziehungen: Wachstumsfaktor – Ertrag verlangte als Voraussetzung die Möglichkeit ihrer Verallgemeinerung, oder der reproduzierbaren Wirkung eines Wachstumsfaktors unter sämtlichen Umweltsbedingungen. Diese Wirkung, in der Größe c der Mitscherlich'schen Gleichung ausgedrückt, wurde von ihm als „Wirkungswert" des betreffenden Faktors bezeichnet und zunächst als konstant angenommen. Die Frage der „Konstanz oder Inkonstanz" dieses Wertes, welche jahrzehntelang den Gegenstand vieler, bedeutender Arbeiten in der ganzen Welt bildete, fand ihre endgültige Antwort im Jahre 1953 auf der Tagung der Deutschen Bodenkundlichen Gesellschaft in Bonn, als Mitscherlich im Rahmen eines Vortrages sagte:

„Ist nämlich ein zweiter Wachstumsfaktor auch in verhältnismäßig geringerer Menge vorhanden, so daß der mit dem

ersten Wachstumsfaktor erreichbare Höchstertrag A verhältnismäßig niedrig ist, dann ist der Wirkungsfaktor c dieses ersten Wachstumsfaktors höher; er nimmt ab, je günstiger sich alle anderen Wachstumsfaktoren gestalten, je höher also der Wert A wird, und das wohl wieder logarithmisch, bis er sich so allmählich einer minimalen Höhe nähert und dann gleich bleibt, also „*konstant*" ist. Wenn wir so bei Gefäß versuchen alle anderen Wachstumsfaktoren so günstig wie nur möglich gestalten oder bei den Feldversuchen eine ausreichende Grunddüngung von allen anderen Nährstoffen geben, so daß der Wert A verhältnismäßig hoch ist, dann ist der Wirkungswert c des einzelnen Wachstumsfaktors in der Tat konstant".

Dadurch war die „bedingte" Konstanz anerkannt, wobei die große Bedeutung des Wirkungsgesetzes zur Lösung von *praktischen* Fragen, wie die der Bodenuntersuchung, unvermindert bleibt. Gerade dieses Problem der Düngerbedürftigkeitsbestimmung unserer Ackerböden, welches vor 50 Jahren – also als Mitscherlich anfing, sich mit ihm zu beschäftigen – die gleiche Bedeutung wie heute für die Forschung besaß, bildete eins der Hauptthemen seiner Lebensarbeit. Wenn Mitscherlich eine „chemische Bodenanalyse" ablehnte und für quantitative Untersuchungen nur den Vegetationsversuch für geeignet hielt, so war das aus dem Grunde, weil er durch eigene, sehr intensive, chemische Arbeiten zu dieser Überzeugung gelangte. Er hatte selbst eine chemische Methode zur Bodenuntersuchung ausgearbeitet, sie dann aber zu Gunsten der pflanzenphysiologischen aufgegeben. Es wäre aber völlig falsch, ihn als *absoluten* Gegner der chemischen Bodenanalyse anzusehen. Er hielt die chemische Bodenanalyse durchaus für brauchbar, wenn man mittels dieser die sehr nährstoffarmen Böden von den übrigen trennen will. Nur eine weitere Unterteilung der Böden hielt er für nicht mehr möglich. Wie hier, war Mitscherlich auch auf allen anderen Gebieten seiner Tätigkeit stets bemüht, exakt zu arbeiten, gleichzeitig aber die Grenzen der notwendigen und möglichen Genauigkeit zu erkennen. In dieser Hinsicht ist er oft mißverstanden worden.

Ein weiterer Gesichtszug, welcher ihn charakterisierte, war die Verbindung mit Problemen der Praxis, wenn man ihm auch oft gerade den Vorwurf von „Theoretiker" in unberechtigter Weise machte. Mitscherlich vereinigte in hohem Maße Sinn für

Theorie und für Praxis. Die praktischen Landwirte Ostpreußens drückten ihre hohe Achtung für seine Verdienste für die Praxis aus, indem sie eine „Mitscherlich-Gesellschaft" bildeten.

Er besaß auch das notwendige Temperament eines Verfechters für seine Ideen und eine unermüdliche, nur durch den Tod abgebrochene Arbeitskraft. So erklärt sich der Umstand, daß, nachdem er sich im Jahre 1941 in Königsberg emeritieren ließ um dann das Familiengut Kutschlau zu übernehmen, nach dem Kriege, als völlig mittel- und obdachloser Flüchtling in Berlin eintraf, mit 72 Jahren wieder Professor für Kulturtechnik an der Berliner Universität wurde. Mit 76 Jahren ließ er sich abermals emeritieren und besaß den Mut, ein Gut der Deutschen Akademie der Wissenschaften zu Berlin für Forschungszwecke zu übernehmen und dort (1950) ein neues „Institut zur Steigerung der Pflanzenerträge" aufzubauen.

295 wissenschaftliche Arbeiten hat Mitscherlich allein oder in Mitarbeit verfaßt. Sein Hauptwerk „Bodenkunde für Landwirte, Forstwirte und Gärtner" erschien in sieben Auflagen: die erste im Jahre 1905, die letzte 48 Jahre später. Ihm verdanken wir neben seinem „Wirkungsgesetz" viele Untersuchungsmethoden und Apparate, darunter die wichtigsten: die Methoden zur Bestimmung der Benetzungswärme und der Hygroskopizität des Bodens, die Bodenuntersuchung mittels der Extraktion mit Kohlensäuregesättigten Wasser, ein Ausgleichsverfahren zur Ausschaltung des systematischen Bodenfehlers bei Feldversuchen; den Vegetationsdüngungsversuch im Gefäß und im Feld; einen Verdunstungsmesser, ein Bohrgerät zur volummäßigen Probeentnahme aus dem Boden, das berühmte Vegetationsgefäß das unter seinem Namen in die ganze Welt Eingang fand, u.a.m.

Trotz der gewaltigen Forschungstätigkeit war Mitscherlich in seinem Leben auch in anderen hohen akademischen Würden sehr aktiv, so zweimal als Rektor der Albertus-Universität und einmal als Dekan der Philosophischen Fakultät in Königsberg, als Dekan der Landwirtschaftlich-Gärtnerischen Fakultät Berlin, dann als geschäftsführender Sekretär der Königsberger Gelehrten-Gesellschaft und als Präsident der IV. Kommission der Internationalen Bodenkundlichen Gesellschaft.

Seine unermüdliche Tätigkeit wurde in verschiedenster Weise anerkannt: 1901 erhielt er den Preis der Liebig-Stiftung, 1925 wurde er zum Mitglied der Leopoldinisch-Karolinisch-Deutschen

Akademie zu Halle, 1947 zum Mitglied der Deutschen Akademie der Wissenschaften und 1951 zum Mitglied der Deutschen Akademie der Landwirtschaftswissenschaften zu Berlin gewählt. Die Landwirtschaftlichen Fakultäten Kiel, Gießen und Berlin verliehen ihm die Würde eines Dr. h.c.

Seine Arbeiten fanden auch im Auslande hohe Anerkennung. So wurde er: 1928 korrespondierendes Mitglied der tschechoslowakischen Akademie der Landwirtschaften; 1932 auswärtiges Mitglied der schwedischen Landwirtschaftlichen Akademie; 1934 korrespondierendes Mitglied der Agrikulturwissenschaftlichen Gesellschaft von Finnland. Außerdem wurde er zum Mitherausgeber der „Soil Science" und der russischen Fachzeitschrift „Pedology" gewählt.

Mitscherlich's Persönlichkeit ergänzte sich durch eine große Aufgeschlossenheit und Bereitschaft zur Mithilfe. Eine große Anzahl von Schülern und Mitarbeitern haben die glückliche Gelegenheit gehabt, von ihm wissenschaftlich zu lernen und gleichzeitig die Güte seines Charakters zu schätzen.

Für die kommenden Generationen bleibt Mitscherlich durch seine Arbeiten und sich daraus ergebenden Anregungen ein lebendiges Bild. An sie adressierte er sich im Jahre 1947, als er die fünfte Auflage seiner „Bodenkunde" herausgab und in ihrem Vorwort schrieb:

> „Die Niederschrift dieses Buches habe ich in meinen Flüchtlingsjahren getätigt, als ich die Überzeugung gewonnen hatte, daß der unselige Krieg auch den größten Teil meiner eigensten Lebensarbeit vernichtet hatte: Ein großer Teil meiner besten Schüler ist nicht mehr am Leben, und der größte Teil meiner zahlreichen Arbeiten dürfte kaum noch auffindbar sein. – So gebe ich in dem nachfolgenden Buche gewissermassen mein wissenschaftliches Testament, und es mag mir aus diesem Grunde verziehen sein, wenn ich in ihm noch einmal den größten Teil meiner Lebensarbeit zusammenfasse".

Berlin N. ATANASIU

ZUR BESTIMMUNG DER WASSERDURCHLÄSSIGKEIT BEIM GEWACHSENEN BODEN

von EILH. ALFRED MITSCHERLICH

Paulinenaue

1. EINLEITUNG

So mancher Bodenkundler hat bereits das Bedürfniss empfunden die Wasserdurchlässigkeit beim gewachsenen Boden bestimmen zu können. Es wurden denn auch im Laufe der Zeit verschiedene Methoden hierfür vorgeschlagen, die hier aber nicht einmal genannt, geschweige denn genauer beschrieben, werden sollen. Das Ziel dieser Mitteilung ist bloss eine in letzter Zeit ausgearbeitete Bestimmungsweise anzugeben, die unseres Erachtens viele Vorteile hat und daher neben den älteren Methoden durchaus existenzberechtigt ist.

Wenn man das Problem näher praezisiert, dann liegt also der Wunsch vor, die Wasserdurchlässigkeit eines Bodens in einer bestimmten Tiefe zu studieren. Dabei muss man sich zunächst darüber im Klaren sein, dass

1. das Wasser, welches man dort eindringen lässt, zunächst keineswegs nur senkrecht in den Boden eindringt, wenn auch diese Richtung stets – der Schwerkraft folgend – bevorzugt sein wird. Das Wasser kann also nach jeder Richtung hin kapillar abgeleitet werden, auch wenn beim Einleiten nicht ein eventueller Wasserdruck vorliegen sollte!

2. das Bodenprofil wechseln kann, so dass auf eine gut wasserdurchlässige Erdschicht bald eine ganz undurchlässige folgen kann und umgekehrt auch in jedwedem Übergange;

3. die Durchlässigkeit einer Erdschicht für Wasser von der Weite ihrer kapillaren Hohlräume, als auch von ihrem Hohlraumvolumen und seinem derzeitigen Wassergehalte bedingt wird.

Da man also wissen muss, wie dieses Bodenvolumen unter der Ein-

flusstiefe beschaffen ist, muss es zunächst auf diese Untersuchung ankommen. Wir bedienen uns dazu des seinerzeit von Beutelspacher und mir konstruierten Bohrgerätes das hierfür unumgänglich notwendig ist.

Obgleich dieses Bohrgerät schon im Jahre 1936 genau beschrieben wurde, meine ich aus an mich gerichteten Fragen von Kollegen ableiten zu müssen, dass das Gerät leider noch nicht genügend bekannt ist. An erster Stelle soll hier also das Bohrgerät von neuem in Kürze besprochen werden.

2. DAS BOHRGERÄT

Das Bohrgerät (Abbildung 1A) besteht aus drei Teilen: Dem Bohrrohr (*a*), der Bohrstange (*b*) und dem Bohrschuh (*c*). Das Bohrrohr besitzt eine Länge von 130 cm. Oben ist es zum Einbohren von zwei Handgriffen (*d*) versehen, die mit Gummischlauch überzogen sind. Am unteren Ende ist ein, aus besonders hartem Stahl angefertigter Bohrkopf (*e*), an dem zwei Flügel (*f*) angeschweisst sind, um das Einbohren in das Erdreich zu erleichtern, befestigt.

Der Bohrkopf hat die Weite des Bohrrohres. Die Aussenwandung desselben läuft scharf zu und hat hier einen Durchmesser von genau 35.7 mm, so dass jedesmal ein Erdzylinder von 10 qcm Fläche der Erde entnommen wird. Damit der Boden beim Ausbohren leichter aus dem Bohrrohr herausrutscht, ist das Rohr von 1.5 cm über der Schneide ab um 2 mm weiter ausgebohrt.

Hat man das Bohrrohr soweit in die Erde eingeschraubt, dass der Bohrkopf mit seinen Flügeln im Boden ist, dann legt man von beiden Seiten des Bohrrohres den Bohrschuh (*c*) an, schiebt seine beiden Hälften zusammen, wofür an der einen Hälfte zwei 8 cm lange Stifte, an der anderen zwei entsprechende Hülsen vorgesehen sind, in die die Stifte eingeschoben werden. Nun tritt man die überstehenden äusseren Kanten in die Erde ein. An diesem Bohrschuh, der nun das Bohrrohr gerade durchlässt, wird die Tiefe der Bohrung bestimmt. Hierzu ist ferner am Bohrrohr selbst alle 5 cm eine Skala eingeschnitten, an der man eine mit einer Feder versehene Messingplatte (*g*) auf die gewünschte Höhe bzw. Tiefe einstellen kann. Ist dann diese Tiefe der Bohrung erreicht, so schleift diese Platte auf dem Bohrschuh, so dass weiter ein tieferes Bohren erst dann möglich ist, wenn diese Einstellscheibe entsprechend höher eingestellt wird. So kann man den Boden z.B. von 0 ab alle 5 cm bis zu 130 cm Tiefe

volumenmässig nach und nach entnehmen. Um den Boden nun quantitativ aus dem Bohrrore herauszuholen, bedienen wir uns der Bohrstange (*b*). Sie wird dazu in das Bohrrohr eingeführt, hat oben eine kleine Kurbel (*h*), in der Mitte nach der Rohrwandung zu eine Führungsscheibe (*i*) und unten einen ebenso grossen Schneckenteller (*k*), auf den durch Drehen der Kurbel der ausgebohrte Boden hochgebracht wird, um dann mit der Bohrstange herausgehoben zu werden.

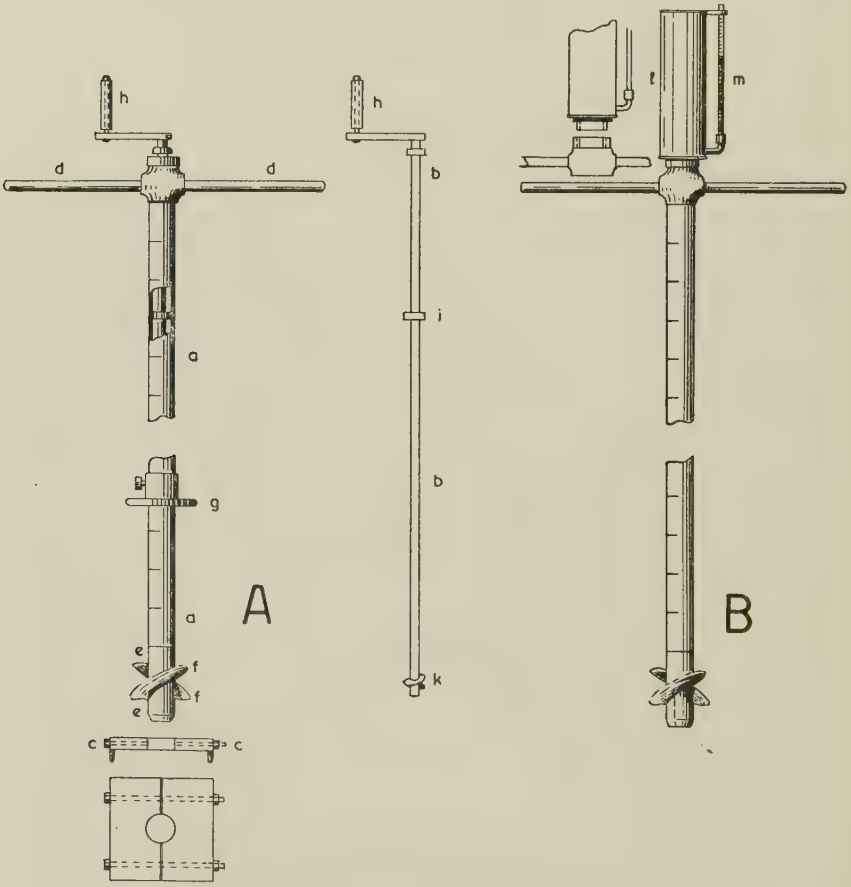


Abb. 1. *A*: das Bohrgerät. *B*: der Apparat zur Feststellung der Wasserdurchflussgeschwindigkeit.

Hat man nun z.B. den Einsteller am Bohrrohre auf 20 cm eingestellt, den ausgebohrten Boden quantitativ beseitigt, dann den Ein-

steller auf 30 cm gebracht und den jetzt ausgebohrten Boden quantitativ entnommen, so war dieser in einem Volumen von 100 cm enthalten. Trocknet man die so entnommene Bodenmasse und bestimmt man ihren Wassergehalt, bestimmt man ferner das spezifische Gewicht des getrockneten Bodens, so hat man das von ihm eingenommene Volumen in ccm, bzw. wenn man dies van 100 ccm abzieht, sein Hohlraumvolumen in Prozenten. So kann man also bereits vieles über die Beschaffenheit des Bodens an erwünschter Stelle erfahren.

3. DER APPARAT ZUR FESTSTELLUNG DER WASSERDURCHFLUSSGESCHWINDIGKEIT IM GEWACHSENEN BODEN

Oben ragt das Bohrrohr 1.5 cm über die Handgriffe heraus (Abbilding 1B). Auf diesem überstehenden Rohr ist ein feines Gewinde eingeschnitten, auf das sich wasserdicht ein 25 cm hoher Wasserbehälter (*l*) aufschrauben lässt, dessen Wassergehalt an einem Standrohre (*m*) beobachtet werden kann. Dieser Wasserbehälter hat einen Durchmesser von 8.0 cm, so dass das Absinken des Wassers um 1 cm = 50 ccm ausmacht. Oder, da das Bohrrohr eine Innenfläche von 10 qcm, das Wassergefäss eine solche von 50 qcm hat, so treten je cm Absinken des Wassers 50 ccm Wasser in den Boden ein, bzw. 5 cm im Rohr gemessen. Zu diesem Durchflussgerät gehört zunächst noch eine ca 150 cm lange Verschlussstange, an der unten ein dicker Gummistopfen befestigt ist, und die in ihrer Mitte nach der Innenwand des Rohres eine Führungsscheibe hat.

Hat man den Boden nun bis zu einer gewünschten Tiefe ausgebohrt, so schliesst man unten das Bohrrohr mit dem Gummistopfen der unten bei seiner Verengung vor der Schneide aufliegt, schraubt, nach Reinigung des Gewindes, den Wasserbehälter auf und füllt nunmehr den ganzen Apparat mit Wasser. Alsdann lüftet man den Stopfen und beobachtet das Aufliessen des Wassers in der Zeiteinheit am Standrohre.

4. UNTERSUCHUNG DER WASSERDURCHLÄSSIGKEIT DES BODENS IN VERSCHIEDENEN TIEFEN

Dazu wird erst an drei, je zwei Meter von der geplanten Versuchsstelle entfernten Stellen der Boden erbohrt und festgestellt, wie in

den nächsten 20 cm unter der geplanten Wasserauslaufhöhe, sein Hohlraumvolumen, sein Wassergehalt, seine Hygroskopizität, u.s.w. beschaffen sind. Man bohrt dann das eigentliche Bohrloch bis zur gewünschten Tiefe aus, entnimmt daraus den Boden, verschliesst unten das Bohrgerät, schraubt das Wassergefäss auf, füllt dieses und das Bohrrohr mit Wasser. Nun kann man, noch Öffnen des Verschlussstopfens, die Beobachtungen ausführen. Zieht man die Stange mit dem Verschlussstopfen nicht ganz aus dem Bohrrohr, sondern hängt man sie an dem Wassergefäss an, dann muss man ihr Volumen in der Höhe des Wassergefässes von dem gemessenen Wasservolumen (= ca 2%) in Abzug bringen.

Als Beispiele können einige Ergebnisse mitgeteilt werden, welche teilweise auf dem Gelände des Institutes in Paulinenaue, auf leichtestem Standboden, teilweise auf dem Universitätsgut Berge auf sandigem Lehm gemacht wurden *. Die nachstehend angeführten Beobachtungswerte sind nun die Teilstriche, welche am Wasserstandsrore des Wassergefässes abgelesen wurden. Um sie in mm Wasserhöhe als Ausfluss in den Boden zu erhalten, sind sie noch mit 5 zu multiplizieren.

Beobachtung 1

Paulinenaue: humoser Sand, 20 cm Tiefe, Hygroskopizität: 1.46% Hohlraumvolumen: 38.5 ± 1.1%						
nach Min.	Beobacht. I	Differenz	Beobacht. II	Differenz	Beobacht. III	Differenz
0.5	10	10	18	18	5	5
1	29	19	26	8	12	7
1.5	41	12	34	8	17	5
2	51	10	40	6	22	5
2.5	61	10	46	6	26	4
3	70	9	51	5	30	4
3.5	78	8	56	5	34	4
4	87	9	61	5	37	3
4.5	95	8	66	5	40	3
5	102	7	70	4	43	3
5.5	109	7	75	5	46	3
6	116	7	79	4	49	3
6.5	122	6	83	4	52	3
7	129	7				
7.5	135	6				
8	141	6				

* Für die Durchführung dieser Versuche spreche ich meinen Mitarbeitern: Prof. Dr. N. Atanasiu und Dipl.-Landw. Köpke meinen besten Dank aus.

Beobachtung 2

Paulinenaue: Sand, 40 cm Tiefe, Hygroskopizität: 0.64% Hohlraumvolumen: 19.9 ± 2.5%						
nach Min.	Beobacht. I	Differenz	Beobacht. II	Differenz	Beobacht. III	Differenz
0.5	14	14	10	10	15	15
1	17	3	15.5	5.5	20	5
1.5	22	5	18	2.5	24.5	4.5
2	26	4	20	2	29	4.5
2.5	30	4	22	2	33.5	4.5
3	33	3	24	2	37	3.5
3.5	36	3	25.5	1.5	39	2
4	39	3	27	1.5	42	3
4.5	41.5	2.5	29	2	45	3
5	44	2.5	30.5	1.5	47	2
5.5	46	2	32	1.5	50	3
6	49	3	33.5	1.5	52	2
6.5	51	2	35	1.5	55	3

Beobachtung 3

Berge: sandiger Lehm, 40 cm Tiefe, Hygroskopizität: 5.32% Hohlraumvolumen: 31.9 ± 0.7%								
nach Min.	Beobacht. I	Differenz	nach Min.	Beobacht. II	Differenz	nach Min.	Beobacht. III	Differenz
0	12	0	0	26.5	26.5	1	12.0	
0.5	12	0	0.5	27.0	0.5	2	18.0	18.0
1.0	12	0	1.0	27.5	0.5	3	19.5	
2.0	12	0	1.5	27.5	0.0	4	21.0	3.0
						6	23.0	2.0
						8	24.5	1.5
						10	25.5	1.0
						12	27.0	1.5
						14	28.0	1.0
						16	29.0	1.0
						18	30.0	1.0
						20	31.0	1.0

Man sieht also zunächst, dass die Wasserdurchflussgeschwindigkeit anfangs hoch und etwas unregelmässig ist, dass sie aber mit der Zeit nachlässt, um sich dann auf einen angenähert konstantbleibenden Minimalwert einzustellen.

Bei den Beobachtungen ist ferner festzustellen, dass der Boden in den untersuchten Fällen wesentliche Unterschiede aufweist:

Beob-acht.	Erdtiefe	Hygros-kopizität	Hohlraum-volumen	Durchfluss-geschwindigkeit
1	20—40 cm	1.46%	38.5 ± 1.1%	6.5 — 4.5 — 3.0 = 4.7
2	40—60 cm	0.64%	19.9 ± 2.5%	2.5 — 1.5 — 2.5 = 2.2
3	40—60 cm	5.32%	31.9 ± 0.7%	0.0 — 0.5 — 1.0 = 0.5

Mit der Dichte der Sandlagerung geht trotz der noch nicht halben Hygroskopizität die Durchflussgeswindigkeit stark zurück. Beim Lehm mit der Hygroskopizität = 5.3% ist sie trotz grossem Hohlraumvolumen fast gleich null.

ZUSAMMENFASSUNG

Es wird im Anschluss an das bereits eher entworfene Bohrgerät ein Apparat beschrieben, mit dem die Wasserdurchlässigkeit des gewachsenen Bodens in verschiednen Tiefen studiert werden kann. Danach werden einige damit ermittelte Versuchsergebnisse mitgeteilt.

Unserem seht tüchtigen Werkmeister, Herrn Bischof, bin ich für die ganz besonders schöne und exakte Ausführung der Apparate ausserordentlich dankbar.

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LITERATUR

Mitscherlich, E. H. und Beutelspacher, H., Z. Pflanzenernähr., Düng. u. Bodenk. **44**, 310–315 (1936).

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OBSERVATIONS ON THE BACTERIAL OXIDATION OF CHLOROPHENOXYACETIC ACIDS

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Audus[1] was the first to isolate a bacterium from soil which could decompose 2,4-dichlorophenoxyacetic acid (2,4-D) and he considered that it belonged to the "*Bacterium globiforme* group" of Lochhead and Taylor[9] but originally described by Conn[5]. The presence of a small amount of agar improved the growth of the bacterium which caused the breakdown of 2,4-D when added to a percolator containing soil crumbs or glass wool and a dilute solution of the herbicidal compound; it could also decompose 2-methyl-4-chlorophenoxyacetic acid (MCPA) but more slowly (Audus[2]).

Two organisms were isolated by Jensen and Petersen[8], one of which was similar to that described by Audus and which was shown to decompose both 2,4-D and MCPA, and the other, which decomposed only 2,4-D, was identified as a strain of *Flavobacterium aquatile* (Frankland and Frankland) Bergey *et al.*[4]. Inoculation of herbicide-treated soil with these organisms resulted in an increased CO_2 output and the detoxication of the herbicidal compound.

Stapp and Spicher[11] have isolated from enriched soil an organism similar to the latter, but which differed slightly in certain characteristics so that these authors considered it to be a new species and gave it the name *Flavobacterium peregrinum* n.sp.. When this organism was grown in a liquid mineral salts 2,4-D medium, more CO_2 was evolved than was contained in the side chain and so they concluded that degradation of the 2,4-D molecule involving fission of the carbocyclic ring had occurred. Spicher[10] found that this organism had an optimum growth temperature of 25°C, was killed

below 45°C and decomposed 2,4-D best at a pH value of 7.0–7.5. More than 0.45% 2,4-D was toxic to the organism.

More recently Weeks[13] has examined seven cultures which various investigators had tentatively identified as *Flavobacterium aquatile*, and compared them in their properties to the original description of the type species. The collection was arranged in four groups on the basis of morphology and pigmentation and Weeks concluded that Jensen and Petersen's organism was a strain of *Sporocytophaga congregata*.

Evans and Smith[6] isolated a gram-negative, motile rod which would grow in a 2,4-D mineral salts medium after an initial lag period. From such cultures, these authors detected a phenolic acid which gave similar colour reactions to 2-hydroxy-4-chlorophenoxyacetic acid but a different R_f value and suggested that this acid might be 6-hydroxy-2,4-D. They also isolated from soil another gram-negative, motile organism which grew aerobically in a medium containing up to 0.1% *p*-chlorophenoxyacetic acid. From these cultures they isolated 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol and found these compounds to satisfy the criteria of simultaneous adaptation, but they gave no experimental details.

Finally, Audus and Symonds[3] studied bacterial growth rates and kinetics of detoxication of 2,4-D in a mineral salts medium by cultures of their strain of *Bacterium globiforme*. They found that phytotoxicity persisted long after bacterial growth had ceased, but that eventually it disappeared after a lag period comparable in duration with those observed in soil perfusion experiments. These results were considered to indicate the formation of phytotoxic intermediates in the early stages of 2,4-D breakdown. Some evidence for two such intermediates was obtained in paper chromatography experiments, but no substance was identified and the authors emphasized the preliminary nature of their results.

In the present work, three herbicide-decomposing organisms have been used. The first, which can decompose 2,4-D and *p*-chlorophenoxyacetic acid, is probably a strain of *Flavobacterium peregrinum*, Stapp et Spicher; the second, which can decompose 2,4-D, MCPA and *p*-chlorophenoxyacetic acid, is an *Achromobacter* species and the third is a similar *Achromobacter* species which decomposes only *p*-chlorophenoxyacetic acid.

METHODS

Soils

Air-dried soil was sieved and the 1–3 mm fraction was separated and used in the experiments. Rothamsted allotment soil, a light clay soil (pH value 6.8) and Woburn Park soil, a sandy loam, (pH value 5.0) were the two types used.

Materials

2,4-D, *p*-chlorophenoxyacetic acid and MCPA were obtained commercially, the last compound being treated with charcoal and recrystallised before use. We are indebted to Monsanto Chemicals Ltd., for a gift of 6-hydroxy-2,4-dichlorophenoxyacetic acid and 2-hydroxy-4-chlorophenoxyacetic acid, to Prof. R. T. Williams for a sample of 4-chlorocatechol and to Dr. G. W. K. Cavill for a sample of 6-hydroxy-2,4-dichlorophenoxyacetic acid.

Enrichment cultures

A mixture of soil (1 part) and a dilute (0.005%), aqueous solution (10 parts) of the sodium salt of one of the chlorophenoxyacetic acids was incubated at 25° in either Erlenmeyer flasks or in Roux bottles. From time to time a sample of the supernatant liquid was taken and examined for herbicidal activity.

Biological assay

The method described by Walker [12] was used for detecting herbicides in soils. In the case of liquid cultures, herbicides were detected by putting a sample (2–3 ml) in a Petri dish, then a layer of filter paper and planting 20 mustard or cress seeds on the paper. The dish was incubated at 25°C and examined after 2 or 3 days.

Media

The same mixture of mineral salts used by Jensen and Petersen formed the basis of our media. The sodium salt of a chlorophenoxyacetic acid (100 to 1000 ppm) was added and, when necessary, 2% agar for plates. In some experiments, Difco dried yeast extract (0.01–0.1%) was added to the medium.

Chloride estimation

Ionic chloride in cultures was estimated by Mohr's method using *N*/20 silver nitrate solution and potassium chromate as internal indicator.

Manometric methods

Organisms were collected from agar plate cultures and washed twice with 0.02 *M*-phosphate buffer (pH 6.98) and then suspended in the same buffer. For comparison purposes the nitrogen content of the cell suspension was determined by the micro-Kjeldahl method. The oxygen uptake by 1 ml cell suspension was measured in Warburg manometers at 30 °C. The main cup of the Warburg vessel contained the cells and enough phosphate buffer to give a

final volume of 3 ml, the substrates being added from the side bulb. The centre cup contained 0.2 ml 20% (w/v) aqueous potassium hydroxide.

EXPERIMENTAL RESULTS

Isolation of organisms

Strain 1. A mixture of Rothamsted allotment soil and a 2,4-D solution was incubated at 25°C until herbicidal activity had disappeared. Four further successive doses (50 ppm) of 2,4-D were added and after the disappearance of each dose, streaked cultures were made on to 0.1% 2,4-D agar plates. These successive platings, after 7–14 days incubation, showed gradually uncreasing numbers of yellow colonies which were subcultured and purified by repeated platings on the same medium. The organism so isolated resembled in many respects that described by Jensen and Petersen and identified tentatively by them as *Flavobacterium aquatile*. It differs from the latter, however, in that it produces, on 1% peptone agar, small discrete yellow colonies with no tendency to spread; whereas Jensen and Petersen's strain produces a spreading growth on peptone agar. Also in peptone water containing 0.1% agar, our organism grew as a surface zone and the rate of growth was reduced by the addition of 1% glucose: Jensen and Petersen's isolate was not affected by glucose under these conditions. Consequently, although we have not yet been able to make a closer comparison with Stapp and Spicher's isolate, we consider our organism to be most probably a strain of *Flavobacterium peregrinum n.sp.* (Stapp and Spicher)). Our strain had the following characteristics:

Gram-negative, non-motile non spore-forming rods, occurring singly or in pairs, 0.5 × 1.6 μ in size. Growth on nutrient agar — soft, lemon-yellow flat colonies with smooth shiny surface and entire edges;

peptone water — uniform turbidity;

gelatin stab — thin, filiform growth in stab, and flat pale yellow surface colony with striate margin. Slow liquefaction after 2–3 weeks;

litmus milk — yellow ring of growth at surface, otherwise unchanged.

No acid or gas was formed from lactose, sucrose, glycerol, mannitol dulcitol or glucose and only slight acid formation from maltose after 28 days. It did not form indole nor reduce nitrate.

It grew in liquid media containing up to 0.2%, but not in 0.4% 2,4-D. In shallow liquid cultures containing yeast extract and 0.1%

2,4-D, all the 2,4-D was decomposed in 12–16 days at 25°C. The organism also grew in 0.1% *p*-chlorophenoxyacetic acid agar but not on *o*-chlorophenoxyacetic acid or on MCPA agar.

Strain 2. From an enrichment culture using Woburn soil, which had received a series of 8 successive doses of MCPA (50 to 200 ppm), an organism, capable of decomposing MCPA, was isolated by plating on to MCPA agar. This organism had the following characteristics:

It consisted of Gram-negative, short, non-motile rods, 0.5 × 1.4–1.8 μ, occurring singly or in pairs. Colonies on nutrient agar were creamy white, convex and shiny. The organism grew but produced neither acid nor gas in sucrose, maltose, mannitol, glycerol, glucose or lactose media. In nutrient broth it grew producing diffuse turbidity and a surface pellicle. It did not form indole nor reduce nitrate Litmus milk was unchanged and gelatin was not liquefied.

It grew in liquid media containing either 0.1% MCPA, 2,4-D or *p*-chlorophenoxyacetic acid as the sole carbon source, but in all cases growth was improved by the addition of yeast extract (up to 0.1%). We consider that the organism is an *Achromobacter* species, but much further study will be required before it can be decided whether it is a new species.

Strain 3. Finally, by streaking on to plates of *p*-chlorophenoxy acetic acid agar, another strain of an *Achromobacter* species was isolated from a 2,4-D enrichment culture using Woburn soil. The organism isolated had the following characteritsics:

Gram-negative, non-motile rods (0.6 × 1.2–1.8 μ) occurring singly or in pairs. Colonies on nutrient agar were shiny, circular and entire with a slight concavity. Growth on nutrient agar slant was greyish white. It formed nitrite from nitrate, and did not form indole nor liquify gelatin after 4 weeks. There was no change in litmus milk. It grew but produced neither acid nor gas in mannitol, sucrose, glucose, lactose, maltose or glycerol media. It attacked phenol.

It grew on 0.1% *p*-chlorophenoxyacetic acid agar or liquid media at 25°C, but not on 2,4-D.

Experiments with soil cultures

The time necessary for the disappearance of herbicidal activity in soil cultures varies with conditions and for different compounds. In

general, higher temperatures up to 30°C and better aeration accelerate the decomposition; for example, in moistened soil kept out side, the time for the complete disappearance of 2,4-D (50 mg/kg) was 37 days whereas in a mixture of soil (10 g) and 2,4-D solution (50 ml, 50 ppm) kept in an incubator at 25°C, the time was about 16 days. 2,4-D and *p*-chlorophenoxyacetic acid were found to disappear at about the same rate, but MCPA decomposed more slowly and 2,4,5-trichlorophenoxyacetic acid (25 ppm) only disappeared from soil cultures after 150 days at 25°C. These results are in general agreement with the findings of previous workers.

Effect of aeration on the breakdown of 2,4-D. A mixture of fresh Rothamsted soil (50 g) and 2,4-D solution (100 ml, 55 ppm) was incubated at 25°C in a Roux bottle until herbicidal activity had disappeared (14 days). A second addition of 2,4-D (120 ppm) was made and this disappeared in 7 days. Then a further addition of 2,4-D (200 ppm) was made and the mixture was divided into two parts, one being put into a Roux bottle and the other completely filling a stoppered bottle and both were incubated at 25°C. After 4 days, no herbicidal activity was detected in the Roux bottle, but activity remained in the sample placed in the stoppered bottle even after 27 days further incubation.

A second experiment was carried out by placing one lot of a mixture of sterile soil crumbs (10 g) and 2,4-D medium (60 ml, 250 ppm) in a sterilised stoppered bottle and another lot of the same mixture in a sterilised Roux bottle. Both were given an inoculum from a pure culture of Strain 1, grown on 2,4-D agar, and then incubated at 25°C. At intervals they were examined for herbicidal activity. After 5 days, the 2,4-D had disppeared from the Roux bottle, exposed to air, but not from the stoppered bottle even after 20 days incubation. After 20 days, the contents of the stoppered bottle were transferred to a sterile Roux bottle and incubated further aerobically at 25°C. After 6-7 days more, herbicidal activity had disappeared.

Cross-adaptation experiments with fresh soil. The effect of pretreatment of a soil with one chlorophenoxyacetic acid on the rate of disappearance of related compounds subsequently applied was examined in the following experiment. Three series of soil enrichment cultures in 500-ml Erlenmeyer flasks were prepared in triplicate, each flask containing Woburn soil crumbs

(10 g) and a solution (50 ml) of either 2,4-D, MCPA or *p*-chlorophenoxyacetic acid (50 ppm in each case). They were incubated at 25°C until the herbicidal activity had disappeared when fresh additions of herbicides were made until each had received and decomposed 4 doses (1st dose, 50 ppm; 2nd dose, 100 ppm; 3rd and 4th doses 200 ppm). The soil cultures were then adapted to decompose the herbicidal compounds at maximal rates. The 3 flasks of each series were then separated, one receiving a dose of the same compound as before, and the other two, different compounds, in all cases the dose was 50 ppm. Incubation was continued and the times for the disappearance of the compounds added were noted; the results are given in Table I.

TABLE I

Disappearance of different herbicidal compounds in pretreated soils

First treatment with	Days for complete breakdown of second treatment with		
	MCPA	2,4–D	*p*-Chlorophenoxyacetic acid
MCPA	4	6	8
2,4–D	6	4	6
p-Chlorophenoxyacetic acid	8	7	4

The results show that the maximum rate of decomposition of a given compound is obtained by pretreatment of the soil with the same compound, but pretreatment with any of the three herbicides results in a more rapid decomposition of the other two compounds than would be the case in fresh soils.

Experiments with pure cultures in liquid media

Strain 1 was found to grow in a liquid mineral salts medium containing 2,4-D as the only source of organic carbon. In cultures in 100-ml Erlenmeyer flasks containing 25 ml of 2,4-D (100 ppm) medium, incubated at 25°C, there was complete disappearance of herbicidal activity after 25 days. The addition of 0.1% yeast extract increased the rate of growth, so that even in cultures containing up to 0.1% 2,4-D, all herbicidal activity disappeared in 12–16 days.

In a medium containing 0.05% yeast extract and 50 ppm herbicide, strain 2 decomposed MCPA or 2,4-D completely in 4 days and *p*-chlorophenoxyacetic acid in 23 days.

Liberation of chlorine from 2,4-D by strain 1. A mineral salts medium containing 0.1% 2,4-D and 0.025% yeast extract was prepared and dispensed in 100-ml quantities in 500-ml Erlenmeyer flasks. Some of these were inoculated with strain 1 and others kept as controls and all were incubated at 25°C. Chloride was estimated in samples removed aseptically at intervals and the results are given in Table II.

TABLE II

Chloride content (mg Cl/100 ml) of strain-1 cultures growing on 2,4–D		
Incubation time in days	Uninoculated control	Strain-1 cultures
7	35.8	35.5
19	35.8	36.5
32	37.6	52.2
39	38.0	59.9

The increase in ionic chloride in the culture after 39 days amounts to 245 mg/litre, which is equivalent to 76% of the 2,4-D chlorine. No corrections have been made for any loss of water by evaporation. Similar increases in the ionic chloride content of the medium have been observed in other experiments with cultures in which there has been decomposition of either 2,4-D or of *p*-chlorophenoxyacetic acid. The rate of decomposition of 2,4-D and the resultant liberation of chlorine in ionic form depends largely on efficient aeration as well as on temperature and the presence of adequate amounts of growth factors in the medium.

A number of attempts have been made to detect possible metabolic intermediates in cultures of 2,4-D decomposing bacteria after the chloride analysis has indicated partial breakdown of the 2,4-D. None of these has so far succeeded, and the only substance isolated and identified has been unchanged 2,4-D.

Action of strain 2 on *p*-chlorophenoxyacetic acid. Evidence of a yellow acidic substance has been obtained in the case of either strain 1 or 2 growing in media containing *p*-chlorophenoxyacetic acid.

Strain 2, previously grown in MCPA-containing media, was inoculated into Roux bottles containing 100 ml mineral salts medium and *p*-chlorophenoxyacetic acid (100 ppm) and then these were incubated at 25°C for 5–6 days. The cultures were straw yellow in colour and

the colour was discharged on acidifying with dilute hydrochloric acid. The contents of eight Roux bottles were combined, acidified by the addition of dilute hydrochloric acid and then extracted three times with ether. The colourless ether extracts were washed with a little water and then extracted with a little 5% aqueous sodium hydrogen carbonate which became deep orange in colour. The aqueous layer was separated, acidified with dilute hydrochloric acid, which discharged the colour, and then extracted with ether. The ether solution was dried over anhydrous sodium sulphate, the ether removed and the residue of impure *p*-chlorophenoxyacetic acid, after crystallisation from water showed m.p. 155°C. It is probable that the ether-soluble residue consisted largely of *p*-chlorophenoxyacetic acid together with a very small amount of the unknown acidic substance which was lost during recrystallisation of the *p*-chlorophenoxyacetic acid. The formulation of a yellow coloured salt from the colourless free acid suggests either a lactone or a quinonoid structure, but the identification of this substance requires a much larger quantity of material.

Respiration studies

Evidence of adaptive enzyme formation. Evidence that the ability to oxidise chlorophenoxyacetic acids depends on adaptive enzyme formation was obtained in the following experiment. After an initial passage on peptone agar and MCPA yeast extract agar respectively, cells of strain 2 were grown on peptone agar and another batch of cells on MCPA-yeast extract agar for 5 days at 25°C. The two lots of cells were harvested separately and washed twice with *M*/50 phosphate buffer and resuspended in buffer solution. Rates of oxygen uptake in presence of either MCPA, 2,4-D or *p*-chlorophenoxyacetic acid were then determined in the usual way. The results, plotted in Fig. 1, showed that cells grown in peptone agar do not oxidise any of the substrates whereas MCPA-grown cells absorbed oxygen in presence of MCPA, even more quickly in presence of 2,4-D and scarcely at all with *p*-chlorophenoxyacetic acid. Thus growth in the presence of MCPA is necessary for the development of the enzyme system catalysing the oxidation of this compound. This conclusion was supported by the fact that 2,4-D-grown cells consumed oxygen at about the same rate as MCPA-grown cells with 2,4-D as the substrate but not with MCPA or

p-chlorophenoxyacetic acid (Fig. 2). It is curious that MCPA-grown cells should oxidise both MCPA and 2,4-D whereas 2,4-D-grown cells showed oxygen uptake only with 2,4-D.

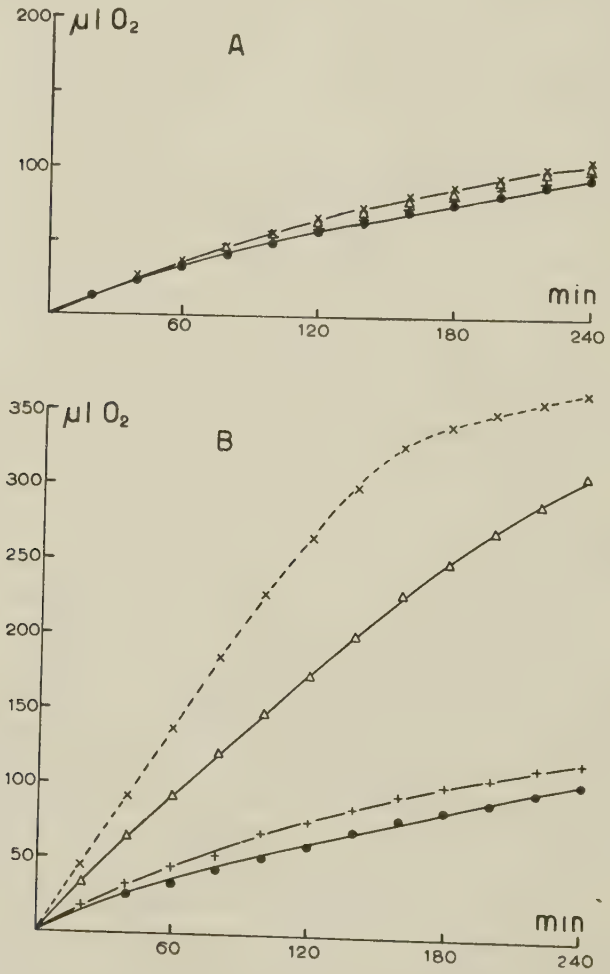

Fig. 1. **A**. Rate of O_2-uptake by washed cells (•——•) of strain 2, grown on peptone, and in presence of 2 µmoles MCPA (△——△), 2,4-D (×——×) or *p*-chlorophenoxyacetic acid (+——+). (The cell suspension contained 0.36 mg N/ml.

B. Rate of O_2-uptake by washed cells (•——•) of strain 2, grown in MCPA, and in presence of 2µmoles MCPA (△——△), 2,4-D (×——×), or *p*-chlorophenoxyacetic acid (+——+). (The cell suspension contained 0 52 mg N/ml).

Experiments with possible metabolic intermediates. Washed cells of strain 1, grown on 2,4-D media for about 2 weeks, were used to study oxygen uptakes with 2,4-D and certain derivatives as substrates. A typical result is shown in Fig. 3 and the amount of oxygen absorbed corresponded to approximately 11 atoms per molecule of 2,4-D. Theoretically one molecule of 2,4-D requires 15 atoms of oxygen for its complete conversion to CO_2, H_2O and HCl as suggested by the following equation:

$$C_8H_6O_3Cl_2 + 15O \rightarrow 8\,CO_2 + 2H_2O + 2\,HCl.$$

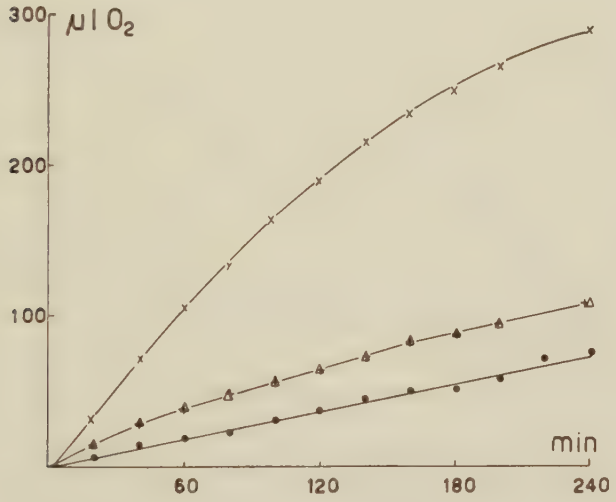

Fig. 2. Rate of O_2-uptake by washed cells (•——•) of strain 2, grown on 2,4-D, and in presence of 2 µmoles MCPA (△——△), 2,4-D (×——×) or *p*-chlorophenoxyacetic acid (+——+). (The cell suspension contained 0.32 mg N/ml).

In this experiment, there was no oxygen uptake in the presence of *p*-chlorophenoxyacetic acid or 2-hydroxy-4-chlorophenoxyacetic acid and the rate of oxygen uptake was very slightly depressed by 6-hydroxy-2,4-dichlorophenoxyacetic acid. Neither was there any oxygen uptake when 2,4-dichlorophenol was added as a substrate. Thus none of these compounds can be considered as metabolic intermediates of 2,4-D if Stanier's simultaneous adaptation hypothesis holds. Difficulty was experienced sometimes in obtaining

active cell suspensions and as yet no clear explanation of the variations observed can be given. For example, cells which respired only very feebly in the presence of 2,4-D were occasionally obtained. Whether essential factors of the enzyme systems involved were removed by washing, or whether the medium on which the cells were grown was deficient in growth factors has not been established.

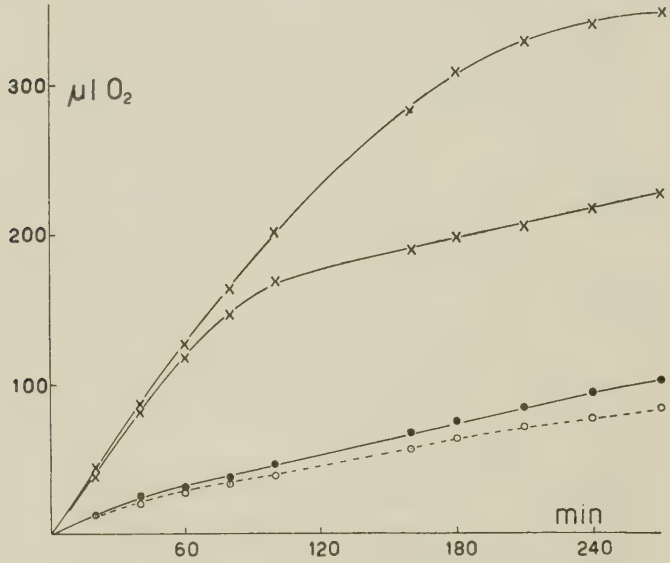

Fig. 3. Rate of O_2-uptake by washed cells (• — — •) of strain 1, grown on 2,4-D media, and in presence of 1 or 2 µmoles 2,4-D (× — — ×), or 1 or 2 µmoles 6-hydroxy-2,4-dichlorophenoxyacetic acid (○ — — ○). (The cell suspension contained 0.55 mg N/ml).

A further experiment was carried out using *p*-chlorophenoxyacetate-grown cells of strain 3 and *p*-chlorophenoxyacetic acid as substrate. The results given in Fig. 4 show that about 100 µl oxygen are absorbed per µmole *p*-chlorophenoxyacetic acid which is equivalent to 9 atoms of oxygen per molecule of herbicide. *p*-Chlorophenol was not oxidised, but 2-hydroxy-4-chlorophenoxyacetic acid, 4-chlorocatechol and catechol were immediately oxidised. Small rates of oxygen uptake were observed also with 2,4-D, 6-hydroxy-2,4-dichlorophenoxyacetic acid and with 3-chlorobenzoic acid. The latter substance was included because in some earlier work (unpublished)

by one of us (N.W.) an increased oxygen uptake has been observed when *m*- or *p*-chlorobenzoic acid was used as the substrate with washed cells of a soil *Corynebacterium* which had been grown in an amino acid medium containing also the same chlorobenzoic acid. The organism did not grow with a chlorobenzoic acid as the sole carbon source so there is no other evidence that the compound is

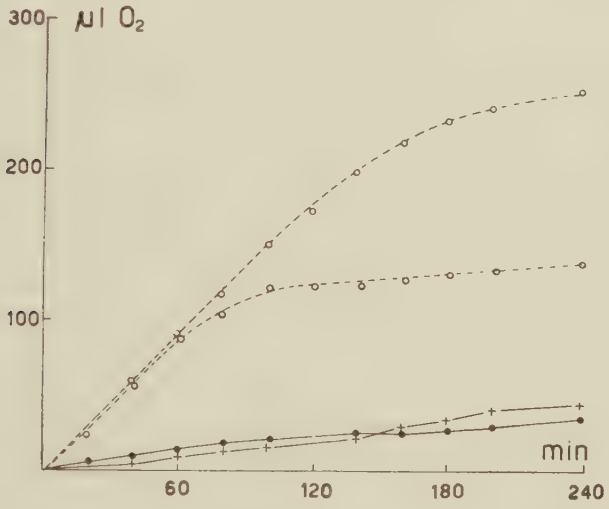

Fig. 4. Rate of O_2-uptake by washed cells (• — — •) of strain 3, grown on *p*-chlorophenoxyacetic acid media, and in presence of 1 or 2 µmoles *p*-chlorophenoxyacetic acid (○ — — ○), or 1 µmole *p*-chlorophenol(+ — — +). (The cell suspension contained 0.39 mg N/ml).

metabolised. Hughes[7] has reported that benzoate-grown *Pseudomonas fluorescens* can oxidise *o*-, *m*- or *p*-fluorobenzoates although these compounds do not support growth of the organisms. These results are mentioned because they indicate the need for caution in interpreting respiration experimental results based upon simultaneous adaptation technique. Consequently although 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol may well be intermediates in the metabolism of *p*-chlorophenoxyacetic acid, as claimed by Evans and Smith, the evidence is rendered less decisive by the fact that the adapted cells oxidise also catechol and the other compounds referred to above.

DISCUSSION

It is now well established that the detoxication of the hormone herbicides in soil is due to the action of micro-organisms and as Jensen and Petersen have pointed out, it is unlikely that naturally occurring organisms would possess constitutive enzymes for metabolising these synthetic compounds. Our experiments with pure cultures of bacteria have shown that the enzymes involved are adaptive since they are possessed only by cells grown in the presence of a given herbicide or, at least, a close analogue. The nature of the enzyme systems concerned cannot be investigated more closely without a considerable supply of such cells.

The other problem relating to the metabolism of chlorophenoxyacetic acids by bacteria is that of the pathway of breakdown, and particularly the fate of the chlorine in the molecule. This is of interest not only because an aromatic chloro-compound is an abnormal nutrient, but also because the covalent bond between chlorine and carbon is generally very stable. In the case of *p*-chlorophenoxyacetic acid, there is good evidence that 2-hydroxy-4-chlorophenoxyacetic acid is an early metabolic intermediate and so by analogy, 6-hydroxy-2,4-dichlorophenoxyacetic acid would be expected to be a metabolite of 2,4-D. But we have failed to obtain any support for this deduction since our 2,4-D-grown cells did not oxidise the 6-hydroxy coumpound.

With strain 3, growing on *p*-chlorophenoxyacetate, a phenol reaction has been detected occasionally in the cultures, which may well have been due to 2-hydroxy-4-chlorophenoxyacetic acid; but when either strain 1 or 2 were grown in *p*-chlorophenoxyacetate media, we have never detected a phenol reaction but instead the cultures became yellow. This colour was due to an ether-soluble, acidic substance which reacted with sodium bicarbonate to form a deep yellow sodium salt. The composition of this substance, which may be an early metabolic product, remains to be investigated.

That the bacterial oxidation of chloro-phenoxyacetic acids does not go completely to CO_2 and H_2O is suggested by the results of chloride analysis, only 76% of the chlorine being accounted for as ionic chlorine. Moreover, the oxygen uptake with 2,4-D corresponded to 11 atoms of oxygen per molecule of 2,4-D and to 9 atoms of oxygen per molecule of *p*-chlorophenoxyacetic acid. Theoretically, one

molecule of 2,4-D, for example, would require 15 atoms of oxygen for complete oxidation. These results suggest that a small fragment of the molecule remains unattacked and this probably contains chlorine and a small number, perhaps 2 or 3, of carbon atoms. The problem of the identity of this postulated residual substance will be investigated further.

SUMMARY

Three bacterial strains, one of *F. peregrinum* (Stapp and Spicher) and two Achromobacter strains, have been isolated from soil and shown to decompose either 2,4-D, MCPA or *p*-chlorophenoxyacetic acid. Aerobic conditions are essential for the bacterial decomposition of 2,4-D. Pretreatment of soil with one of the three chlorophenoxyacetic acids accelerated the rate of breakdown of either of the other two. In a liquid medium, growth of the *F. peregrinum* strain caused breakdown of 2,4-D and liberated 76% of the chlorine in 2,4-D in ionic form. An unknown acidic substance, colourless in acid solution but forming a yellow sodium salt has been detected in cultures of *F. peregrinum* or an MCPA-decomposing Achromobacter strain growing in *p*-chlorophenoxyacetate medium. The bacterial oxidation of chlorophenoxyacetic acid herbicides was attributed to adaptive enzyme formation. Respiration experiments showed that the oxidation of 2,4-D or of *p*-chlorophenoxyacetic acid is incomplete. 4-Chloro-2-hydroxyphenoxyacetic acid and 4-chlorocatechol may be metabolic intermediates in the case of *p*-chlorophenoxyacetic acid, but no intermediary metabolites have as yet been established for 2,4-D.

ACKNOWLEDGEMENT

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REFERENCES

1 Audus, L. J., The Biological detoxification of 2,4-dichlorophenoxyacetic acid in soils; isolation of an effective organism. Nature **166**, 356 (1950).
2 Audus, L. J., The biological detoxification of hormone herbicides in soil. Plant and soil **3**, 170 (1951)
3 Audus, L. J. and Symonds, K. V., Further studies on the breakdown of 2,4-dichlorophenoxyacetic acid by a soil bacterium. Ann. Applied Biol. **42**, 174 (1955).
4 Bergey, *et al.*, Manual of Determinative Bacteriology. 1st ed., 1923, p. 100.
5 Conn, H. J., N.Y. Agr. Exp. Stat. Tech. Bull. No. 138 (July, 1928).
6 Evans, W. C. and Smith, B. S. W., The photochemical inactivation and microbial metabolism of the chlorophenoxyacetic acid herbicides. Bioch. J. **57**, XXX (1954).

7 Hughes, D. E., The effect of mono-fluoro-benzoic acid on adaptation to benzoic acid in *Pseudomonas fluorescens*. Riass. Comm. VI. Congr. Intern. Microbiol. **1**, 141.
8 Jensen, H. L. and Petersen, H. I., Decomposition of hormone herbicides by bacteria. Acta Agric. Scand. **2**, 215 (1952).
9 Lochhead, A. G. and Taylor, C. B., Qualitative studies of soil micro-organisms I. General introduction. Canadian J. Res. C, **16**, 152 (1938).
10 Spicher, G., Beiträge zur Kenntnis der Wirksamkeit des 2,4-D-Zersetzers *Fl. peregrinum*. St. et Sp. Zentr. Bakteriol., II Abt. **108**, 225 (1954).
11 Stapp, C. and Spicher, G., Untersuchungen über die Wirkung von 2,4-D im Boden. IV Mitteilung: *Flavobacterium peregrinum* n. sp. und seine Fähigkeit zum Abbau des Hormones. Zentr. Bakteriol., II Abt. **108**, 113 (1954).
12 Walker, N., Preliminary observations on the decomposition of chlorophenols in soil. Plant and Soil **5**, 194 (1954).
13 Weeks, O. B., *Flavobacterium Aquatile* (Frankland and Frankland) Bergey *et al.*, type species of the genus Flavobacterium. J. Bacteriol. **69**, 649 (1955).

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UTILIZATION OF FERTILIZER NITROGEN BY PLANTS IN SOILS OF DIFFERENT STRUCTURE

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The influence of structure on plant yields has been investigated by a number of workers, and reviewed by P a g e and B o d m a n [3]. In a paper by the present author [1] it was assumed that one of the reasons for the influence of structure on plant growth is the differential availability of nitrogen. In a soil having small aggregates, and thus a small volume of air filled pores the oxygen supply is probably deficient for satisfactory oxidation of nitrogen compounds.

The purpose of the pot experiments described here was to study the influence of structure on the consumption of soil nitrogen. The nitrogen was supplied in mineral and organic form, and the yields obtained were measured.

MATERIALS

The soil used in the experiment was a loess sandy loam, containing 16% $CaCO_3$ and 0.46% organic matter, from Gilat in the Negev region. The whole bulk of soil was passed through a 2-mm sieve and mixed. As the soil is naturally very poorly aggregated, it was treated with a soil conditioner and thus aggregates were formed. The conditioner used was a VAMA type in powder form. 25-kilogram lots of soil were mixed with 50 g of conditioner and subsequently small portions of water were added with mixing, until the pulverized soil cohered into small granules. The aggregated soil was air dried and the different lots remixed. One third of the total bulk of soil was placed into pots, and the remaining two thirds were crushed to pass a 2-mm sieve. One half of the soil thus prepared was placed in pots, and the rest was crushed again to pass a 0.5-mm sieve, and then also filled into pots. Thus 21 Mitscherlich pots were filled with soil of each aggregate size class, each containing 6 kg of air-dry soil. In order to simplify the tabulation of results, the soil which was not crushed will be called the coarsely aggregated soil, the portion which passed a 2-mm sieve the medium aggregated soil, and that part which passed a 0.5-mm sieve, finely aggregated soil.

The fertilizers used were: Superphosphate, containing 44 per cent of P_2O_5; potassium sulphate, containing 54 per cent K_2O; and ammonium nitrate, containing 35 per cent N.

Organic nitrogen was supplied by an oil seed meal (after extraction of oil) containing 6.4 per cent N.

ANALYTICAL METHODS

The water-stable aggregates analysis, pH measurements, conductivity measurements of the soil paste, particle density and air permeability measurements were performed according to Richards [4]. The air permeability was measured in air-dry soil samples and separately in samples which were wetted to their "field capacity". Total porosity was calculated from particle density and from the bulk density, which was evaluated from direct measurements of the volume and weight of soil in pots. The noncapillary porosity and field capacity were found as described elsewhere [1]. Nitrates were determined in water extracts by the phenol-disulfonic method [6]. Total nitrogen in the plants and the seed meal by Kjeldahl method. The statistical analysis of variance was calculated according to Snedecor [5].

EXPERIMENTAL

Some characteristics of the three differently aggregated soils are given in Table I. The crushing and sieving procedures induced considerable differences in water-stable aggregates sizes in the various soils. Also differences in noncapillary porosity and air permeability may be observed.

TABLE I

Analysis of soil before fertilization	Coarsely aggregated	Medium aggregated	Finely aggregated
Size of water-stable aggregates, % { > 2 mm	42,5	7,3	0.0
Size of water-stable aggregates, % { > 0,25 mm	84,7	78,0	19.7
Field capacity, %	18,7	18,9	23,6
Pore space, %	59,2	58,6	46,8
Non-capillary porosity, %	39,4	38,2	14,0
Air permeability { in dry soil, darcys	5,2	4,3	2,4
Air permeability { in wet soil, darcys	2,2	1,3	0,6
pH	7,83	7,83	7,83
Soil paste conductivity, millimhos/cm.	0,80	0.79	0,81
NO_3, ppm.	49	51	52

All 63 pots received the same phosphorus and potassium fertilization, namely 3.0 g of the superphosphate and 0.50 g of the potassium sulfate per pot, which quantities were considered to be adequate for good plant growth.

The nitrogen fertilization was given in a factorial design. The nitrogen was supplied in the mineral form on four levels, and on the same number of levels in organic form. The three treatments, coarsely, medium and finely aggregated soil received nitrogen applications on all levels, each in three replicates. The applications per pot were:

Control, no nitrogen applied.

0.60 g ammonium nitrate	20 g seed meal
1.20 g ,, ,,	40 g ,, ,,
2.40 g ,, ,,	80 g ,, ,,

The fertilizers were mixed with the upper half of the soil in each pot.

Wheat was sown on December 19, 1954, and grown in a greenhouse. The pots were watered to full field capacity and the soil was kept approximatly at this moisture content by frequent watering. In every pot, 10 plants were grown.

On December 25, 1954 the plants emerged.

In the first few weeks the growth of plants in the pots was recorded by measuring the total leaf area. For the purpose of expressing the leaf area, the length of the leaves and their width at the base were measured, and the area calculated as if the leaf had a triangular shape. In table II the measurements at three dates are given. Analysis of variance for the measurements on each date showed that F for treatments at all fertility levels is significant on the 0.01 level. However, the F due to structure differences is significant only on the 0.05 level, and due to nitrogen levels on the 0.01

TABLE II

Measurement of leaf area (cm^2) during the first weeks of wheat growth

Date of measurement	December 31			January 7			January 19		
Treatment	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
Control .	233	195	187	511	483	506	1707	1680	1651
NH_4NO_3									
0.60 g . .	220	204	175	534	506	506	1881	1950	1883
1.20 g . .	193	191	186	523	518	521	2039	2199	2065
2.40 g . .	189	179	147	479	493	460	1498	1812	1779
Seed meal									
20 g .. .	203	175	166	524	473	437	1721	1882	1710
40 g . .	187	186	183	411	470	411	1361	1420	1161
80 g . .	176	170	150	365	326	330	1011	841	804

level. Interactions between structure and nitrogen level were found not to be significant.

As may be seen from data in Table II, at the time of the first measurements (6 day old plants) the plants were smaller in the finer aggregated soils than in the coarser ones, at all nitrogen levels. It appears that the poorer structural conditions in the finer aggregated soils influenced the growth of plants in its first stage. It is of interest to notice that at the same time the added nitrogen had some bad effect on growth. It may be assumed that at this stage the salt effect of the fertilizers was stronger than the effect of nutrient supply.

At later measurements the influence of structure on growth was not so eminent. However, better growth was produced in the fertilized pots than in the control, up to a certain level of added nitrogen.

On March 24, 1955 the plants were harvested. At that time more than half the plants were in bloom. The fresh weight was recorded, after which the wheat was dried in an oven and the oven-dry plants were weighed. In Table III the fresh and dry weights are tabulated.

TABLE III

Weight of plants at harvest and nitrogen accumulated in plants (g per pot)									
	Fresh weight			Dry weight			Nitrogen accumulated		
Treatment	Coarse	Medium	Fine	Coarse	Medium	Fine	Coarse	Medium	Fine
Control .	101.6	58.6	52.9	20.8	14.0	13.5	0.28	0.15	0.14
NH_4NO_3									
0.60 g . .	111.6	109.2	103.9	26.2	25,8	22.3	0.37	0.37	0.33
1.20 g . .	149.3	129.5	117.4	28.2	27.9	25.1	0.53	0.54	0.50
2.40 g . .	145.1	145.9	140.0	26.5	25.6	25.6	0.66	0.71	0.65
Seed meal									
20 g . .	160.2	114.5	96.1	29.4	24.0	19.9	0.77	0.64	0.49
40 g . .	156.7	109.8	84.9	28.7	23.0	17.5	0.78	0.60	0.45
80 g . .	54.7	34.2	22.2	11.6	7.9	5.1	0.31	0.21	0.13

The numbers represent mean weight per treatment. Analysis of variance showed that the differences for treatments are significant on the 0.01 level. In a more detailed analysis it was revealed that the differences due to structure are significant only on the 0.05 level, and due to nitrogen levels on the 0.01 level. Interactions of structure and nitrogen levels are not significant.

Nitrogen was determined in the dried wheat and the amount of nitrogen accumulated by the green parts of plants per pot was

calculated. These results are also given in Table III. The analysis of variance showed the same significance for nitrogen percent as for yields.

On examination of Table III it becomes obvious that soil aggregation had a strong effect on yield in pots which did not receive any additional nitrogen. However, by addition of mineral nitrogen to pots, the differences in yield of plants growing in soils of different aggregation tended to become smaller.

The picture is quite different in pots which received organic nitrogen. There the relative differences in yields due to differences in structure persist on all levels.

It is interesting to note that yields were higher for each additional portion of mineral nitrogen, except in the coarsely aggregated soil, where the second portion already gave the maximum yield. Likewise, the largest portion of the seed meal resulted in an appreciable depression of yield.

The accumulation of nitrogen by plants was consistent with the amounts of mineral nitrogen applied. The influence of structure differences in this case was evident in the control pots, nearly disappeared with the first portion of nitrogen applied and was not appreciable at all for the higher levels.

In the treatments which received organic nitrogen there was an appreciable accumulation of nitrogen in plants on the first two levels, and on the third level the amounts accumulated were similar to those in the control pots, although the yields were lower than in the control.

An additional calculation was performed in order to show more clearly the influence on yields of nitrogen additions in the mineral and organic forms, to soils of different aggregation. The dry matter yield obtained from the coarsely aggregated soil was in each case assumed to be 100 per cent and the relative dry yield from the finely aggregated soil, on the same nitrogen level was calculated according to it. The result is given in the form of a diagram in Figure 1. It is clear from the diagram that the influence of aggregation on wheat yields progressively decreases as the mineral nitrogen supply is more abundant, whereas it increases with larger portions of seed meal applications.

The pH of the soil paste and the conductivity of the saturation extract of soils after harvest were measured. The results are tabulated

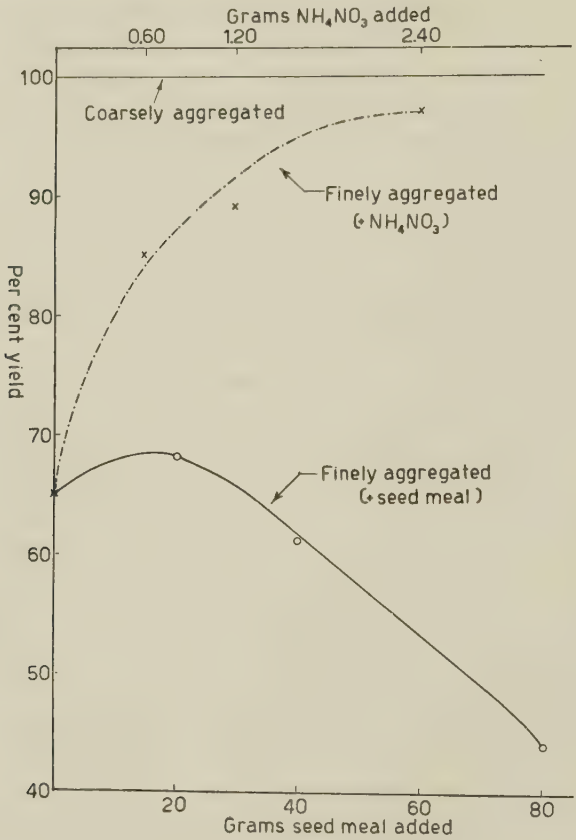

Fig. 1. Per cent of yield in the finely aggregated treatments (The yield in the coarsely aggregated = 100%)

in Table IV. Appreciable changes in conductivity and pH may be seen in the treatments which received higher applications of seed meal.

Aggregation was again measured in the pots after harvest. The analysis of water-stable aggregates is presented in Table V. It may be seen from the table that in the coarsely and medium aggregated treatments no appreciable changes in aggregation took place as a consequence of plant growth. In the finely aggregated soil some improvement of aggregation occurred. However, the differences in structure between the treatments are highly significant after harvest also.

TABLE IV

pH and conductivity of the soils after harvest

Treatment	pH			EC_{25} (millimohs)		
	Coarse	Medium	Fine	Coarse	Medium	Fine
Control	7.95	7.98	7.89	0.56	0.65	0.68
NH_4NO_3						
0.60 g	7.84	7.94	7.91	0.42	0.58	0.51
1.20 g	7.97	7.98	7.85	0.41	0.43	0.52
2.40 g	7.96	7.88	7.83	0.48	0.54	0.67
Seed meal						
20 g	7.84	7.88	7.97	0.71	0.64	0.68
40 g	7.41	7.45	7.48	1.84	1.91	2.00
80 g	7.35	7.31	7.34	2.19	2.24	2.73

TABLE V

Water-stable aggregates in the soils after harvest (%)

Treatment	Coarse		Medium		Fine	
Size of aggregates	> 2 mm	> 0.25 mm	> 2 mm	> 0.25 mm	> 2 mm	> 0.25 mm
Control	38.2	72.7	7.9	76.5	1.1	36.4
NH_4NO_3						
0.60 g	37.4	67.2	5.6	73.7	3.7	32.8
1.20 g	37.9	68.5	6.2	79.9	2.7	32.0
2.40 g	41.3	65.3	4.7	74.5	2.4	37.3
Seed meal						
20 g	38.4	69.1	8.0	73.0	5.6	37.1
40 g	43.8	74.4	6.1	72.5	5.6	35.3
80 g	41.2	74.6	7.6	76.5	4.0	32.1

DISCUSSION

These pot experiments were performed in order to study 1) whether additions of nitrate nitrogen to poorly aggregated soils will raise their yields to the level of those containing larger aggregates; and 2) mineralization of nitrogen from organic forms in soils of different aggregation and its availability to plants.

In the control pots the poorly aggregated soil produced a much lower yield than the one containing large aggregates. In a similar experiment [2] it was found that oxygen diffusion rates were lower in the finely aggregated soil than in the coarsely aggregated one. Accordingly it may be assumed that aeration of the finely aggregated

soil was lower than in the coarsely aggregated. Differences in non-capillary porosity and air permeability found in this work, tend to confirm this assumption. It is recognized that aeration conditions are responsible for differences in plant yields [3]. The soil used in this experiment had an extremely low organic matter content. Thus it seems that a slow mineralization of nitrogen in finely aggregated soil could not play an important role in reducing yields. Soil aeration probably had a strong effect on root development and nitrogen assimilation. However, an abundant supply of nitrogen in mineral form nearly overcame the negative influences of poor aggregation. It may be concluded that under the conditions of this experiment, good plant growth could be obtained even in a finely aggregated soil by supplying mineral nitrogen abundantly. No conclusions as to nutrient supply in general can be drawn, as other nutrients were supplied equally and fully to all treatments.

Analysis of the result of that part of experiment in which organic nitrogen was added to the soil, show that in the case of the first two levels of added nitrogen, the plants responded better and gave higher yields in the coarser aggregated soils than in the fine one. Presumably the conditions in the fine aggregated soil did not allow as high a nitrogen mineralization rate as in the coarser ones, and thus the nitrogen supply to plants was possibly better in the coarsely aggregated soil.

The soils which received the highest amount of added organic matter gave lower yields than the controls. In this case it seems that the large supply of decomposable organic matter induced strong microbiologic competition for nutrients.

It may be concluded that additions of organic nitrogen to a soil of poor aggregation might somewhat raise yields, but that the increase is limited to conditions of relatively slow nitrogen mineralization.

As in a previous paper [1] it should again be stated that in pot experiments with fertilizers, changes in structure may induce considerable differences in plant yields obtained.

SUMMARY

A pot experiment was performed to study the influence of soil aggregation on wheat growth and consumption of nitrogen. Three aggregate size classes of a loess soil were prepared and were fertilized on four levels of nitrogen in two forms (mineral and organic).

Potassium and phosphorus were added equally to all treatments.

The original, poorly aggregated soil was treated with a soil conditioner and crushed subsequently to obtain the three aggregate size classes.

The coarsely aggregated soil produced higher yields than the finely aggregated one. However, additions of mineral nitrogen tended to eliminate the yield differences. Additions of organic nitrogen, on the other hand, rather enlarged the yield differences between the coarsely and finely aggregated soils.

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REFERENCES

1 Hagin, J., Influence of soil aggregation on plant growth. Soil Sci. **74**, 471–478 (1952).
2 Monselise, S. P. and Hagin, J., Influence of soil aggregation on the rooting of carnation cuttings. Plant and Soil **6**, 245–250 (1955).
3 Page, J. B. and Bodman, G. B., The effect of soil physical properties on nutrient availability. In: Mineral Nutrition of Plants, p. 133–166. The University of Wisconsin Press (1951).
4 Richards, L. A. (editor), Diagnosis and Improvement of Saline and Alkali Soils. USDA Agric. Handbook No. 60 (1954).
5 Snedecor, G. W., Statistical Methods. Iowa State College Press (1950).
6 Snell, F. D. and Snell, C. T., Colorimetric Methods of Analysis, II. D. Van Nostrand Co. (1949).

Plant and Soil VIII, no 1 August 1956

THE PREPARATION OF MICROTOME SECTIONS OF UNALTERED SOIL FOR THE STUDY OF SOIL ORGANISMS IN SITU

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INTRODUCTION

In attempting to discover the part played by nematodes in the soil the part played by other organisms should not be neglected. The question is, however, in what way can they be included in the investigations.

As the chief aim is to try to form a picture of the spatial distribution in soil of organisms such as bacteria and fungi, plate cultures do not help. Such problems can only be studied by the microscopical examination of soil which has been distorted as little as possible.

Kubiëna [3] attempted to make investigations of this kind by thoroughly drying samples of soil which were subsequently embedded in resins or plastics immiscible with water. With such treatment all living animal tissue shrivels to such an extent that the organisms become unrecognizable. Haarløv and Weis-Fogh [1] described a method which does not involve drying the soil samples. Sections 500 μ or more in thickness are prepared, the sand grains being pushed aside by the knife during cutting. This technique is not quite suitable for detailed examination of nematodes, bacteria and fungi. By the use of the technique described below it is possible to cut sections (7.5 μ or more in thickness), even of coarse sandy soils, without disturbing the original positions of the soil particles. In addition the organisms are not dehydrated and can be readily observed after staining. Although this technique is still in an early stage of development and far from faultless, it has already given some useful results.

METHODS

Soil samples, about 55 ml in volume, are taken in the field by means of steel cylinders measuring 4.5 cm in length and 4.5 cm in diameter. A strong knife is used to dig out the cylinder containing the soil sample. The cylinder containing the soil sample is transported to the laboratory in a plastic bag where the sample, still in the cylinder, is held at a temperature of — 10°C for a period of at least 12 hours. The temperature of the samples is then increased slightly, but not sufficiently to allow thawing to take place, and a small cylindrical sample is removed by means of a steel sampler as used by Haarløv and Weis-Fogh. (Fig. 1 and 2).

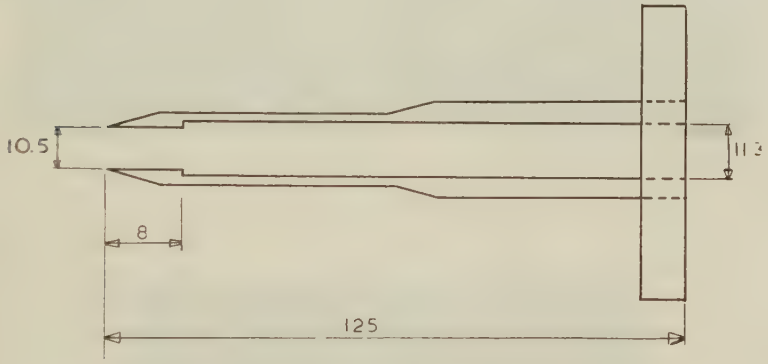

Fig. 1. Steel sampler, after Haarløv

When the frozen soil is extruded from the cylinder, it is cut into discs 4.5 mm thick which fit into holes in blocks of a plastic material. (Fig. 2). Each block containing a disc of frozen soil is then wrapped in a piece of copper gauze to prevent loss of soil from the samples when they are slowly immersed in a solution of gelatin having a temperature of 35 C. They are first immersed in a 5% solution of gelatin in water. This treatment is followed by similar treatment in 10, 15 and 20% gelatin solutions. Each immersion takes 1 2 hours and is carried out with the aid of an apparatus which makes use of the hour hand of an alarm clock and a pulley system (Fig. 3). On completion of this process of impregnation with gelatin, the beaker containing the soil samples and the gelatin is cooled. The blocks containing the soil samples are cut out of the excess gelatin

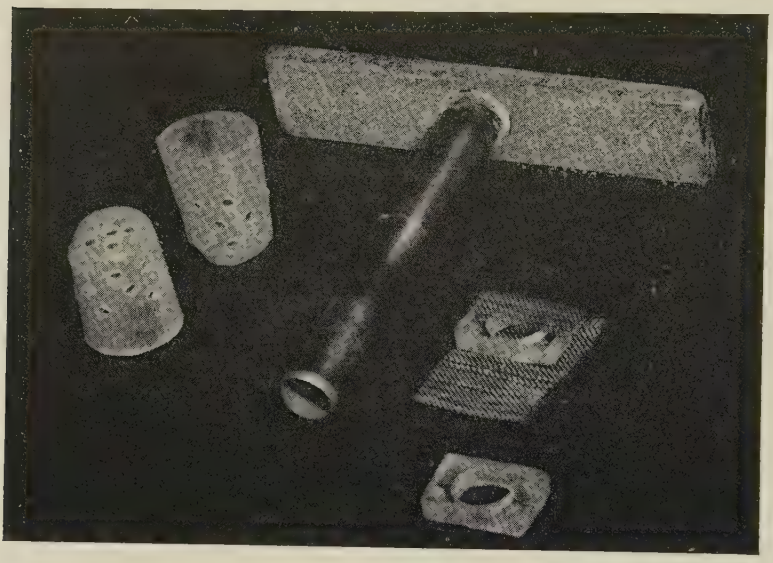

Fig. 2. Steel sampler, plastic tubes and blocks.

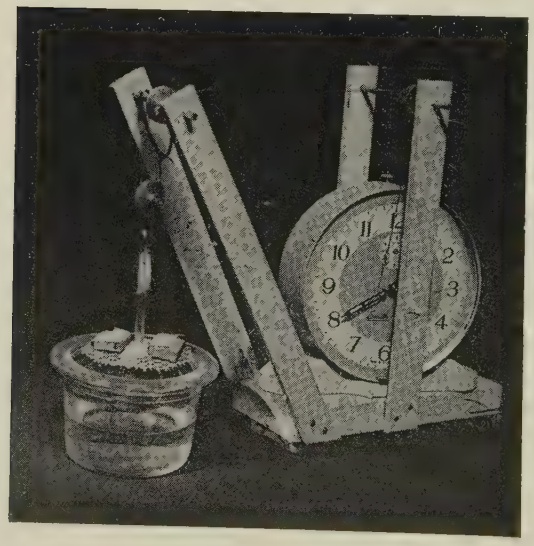

Fig. 3. Explanation in text.

and placed in a considerable volume of 10% formalin (*i.e.* 1 vol. of 40% formalin to 3 vols. of water). The gelatin should not swell when placed in this solution. The formalin solution must be changed at least once during the period of fixing which will take a minimum of seven days.

The blocks are now unwrapped, the samples taken out and cut in half. Both halves of each block are placed in a perforated plastic tube (polythene) (Fig. 2) and immersed in hydro-fluoric acid (50%). The plastic vessel containing the acid and samples is stored in a cool place at a temperature of 15°C or less. It takes from one to seven days to dissolve all sand particles depending on the nature of the soil. The progress of this process is checked by periodically bisecting a half block. When the samples are considered to be free from sand they are washed in water to remove the acid. If it takes too long for the reaction of the washing water to reach a pH of 3.0 or thereabout, a very dilute solution of ammonia may be used as the washing fluid.

The washed half blocks are then again immersed in 15 or 20% gelatin. When cool, cubes are cut from the mass of gelatin, each cube containing a half sample. Gelatin is used to affix these cubes to wooden blocks which can be clamped in a microtome for sectioning of the gelatin cubes.

Before sectioning, the cubes are again immersed in formalin solution for one to two days to fix the gelatin of the second infiltration. This is followed by immersion in methylalcohol (80–90%) until the gelatin is hard enough for cutting, this usually takes 24 hours.

A "Sartorius Universal Mikrotom", in which the original knife is replaced by a home-made holder for safety razor blades, has been used for cutting sections (Fig. 4 and 5). These easily replaced blades are used as it is never certain that all sand particles have been dissolved. "Rembrandt" stainless, hollow ground, blades, 0.1 mm thick, have been found to be the most satisfactory for the purpose.

The sections are cut as thin as possible, mostly at 7.5 or 10 μ. They are transferred, with the help of a brush, to 4% formalin solution (1 : 9) or directly to a slide smeared with Haupt's adhesive.

The sections are aligned on the slides and are firmly pressed by hand beneath several layers of filterpaper. The slides thus treated are placed overnight in a desiccator partly filled with 10% formalin

solution (1 : 3), to ensure good adhesion of the sections to the slides. The sections are now ready for staining.

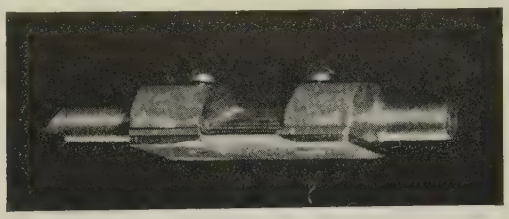

Fig. 4. Razor blade holder.

The best results were obtained with the quadruple staining method of Johansen[2] followed by mounting in Canada balsam, Caedax or Euparal-vert (Plate I and II). The original staining schedule proposed by Johansen has been altered as follows: Safranin, 48 hrs; Methylviolet, 15 minutes; Fast green F.C.F., overnight; Orange G., 30 minutes.

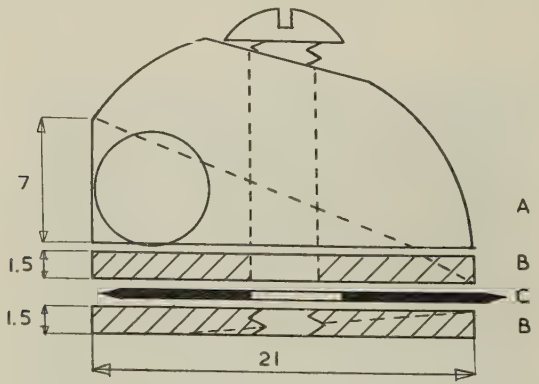

Fig. 5. Razor blade holder. A. Body of brass. B. Steel plate (V2A). C. Razor blade.

It was considered necessary to replace the ethyl alcohol of the staining solutions by methyl alcohol in order to minimise shrinkage.

Trials were made using the following stains: Thionin, Delafield's haematoxylin, Methylene blue in 5% phenol followed by Martius' yellow in water, Giemsa with phosphate buffer, Grenacher's alum-carmine followed by differentiation in 5% ammonium alum, but

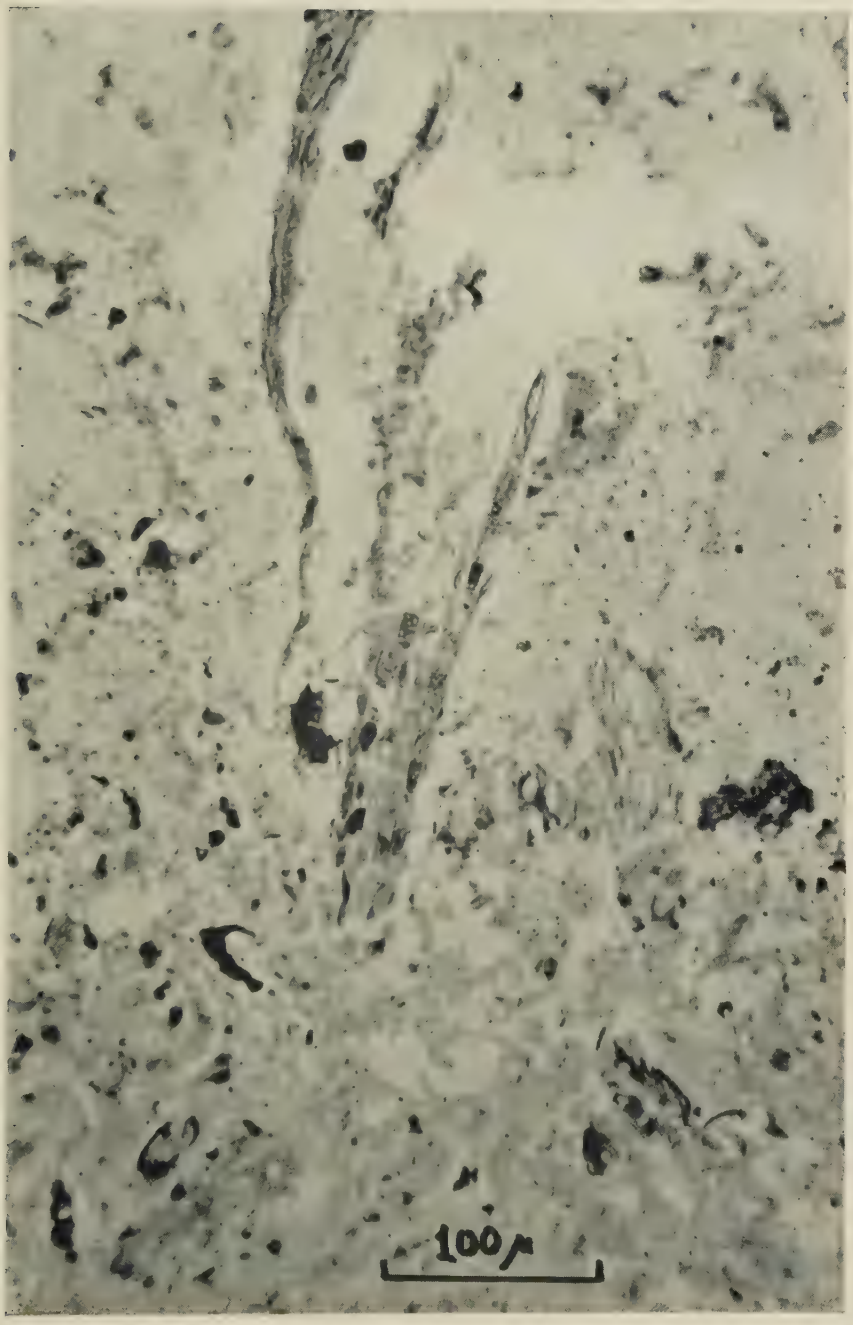

Plate 1. Part of a thin section from the topsoil of an oak forest. The wedgelike objects are dead leaves. (objective 10×, oc. 12.5×)

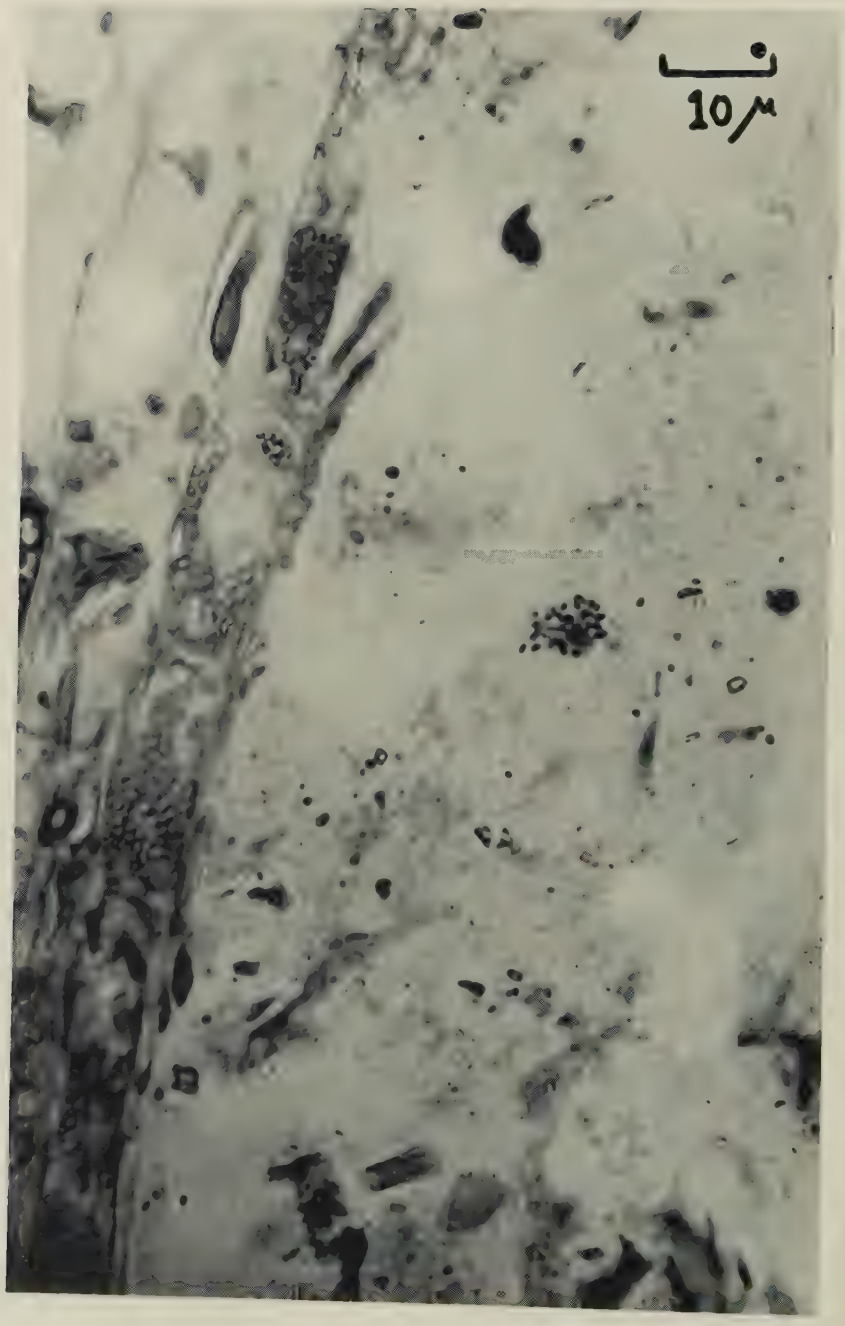

Plate II. Detail of plate I.
The granules visible in the leaf cells presumably are starch grains.
(Gram negative and double refractive in polarised light)
To the right of the leaf a colony of Gram-positive bacteria
(objective 100×, oc. 5×)

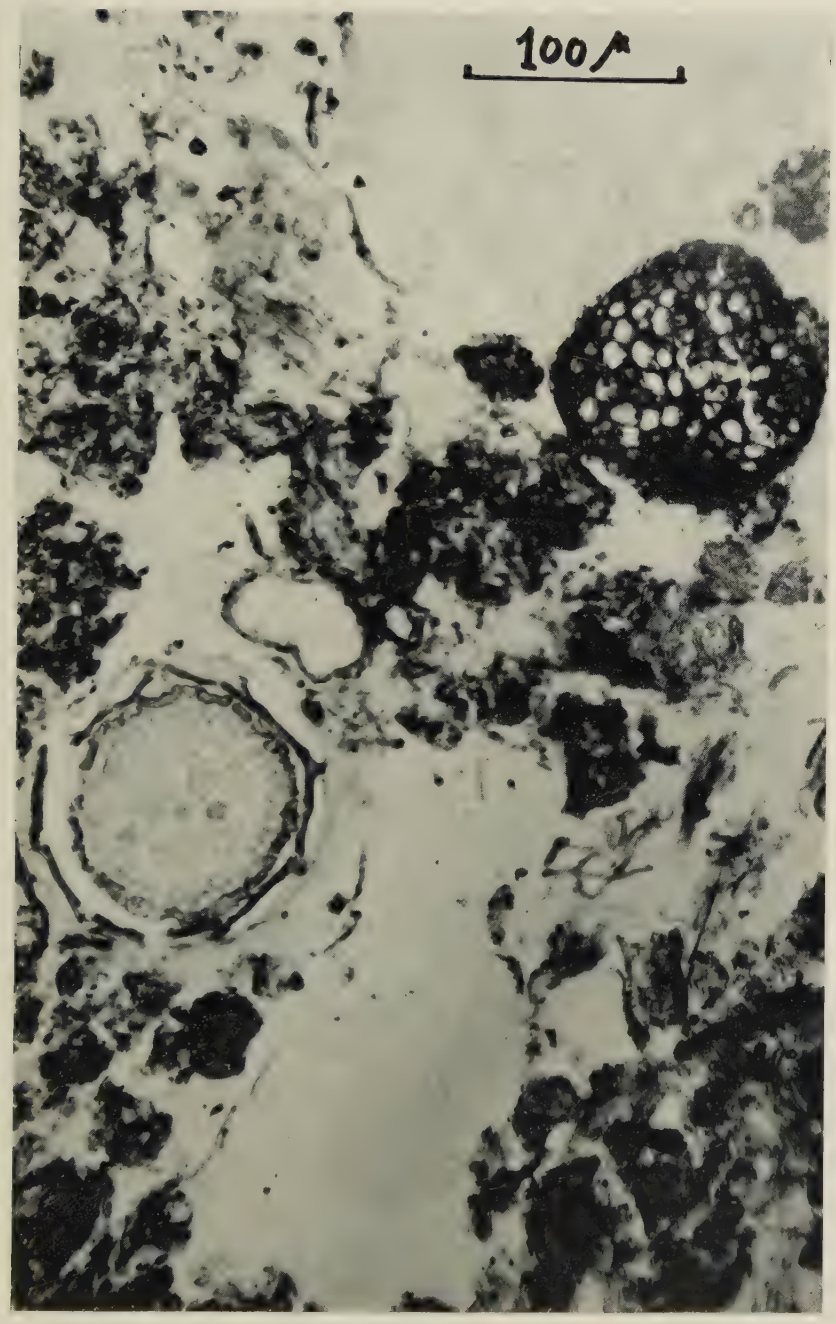

Plate III. Heath soil. At the upper right a sclerotium. At the left a rootlet surrounded by hyphae. (objective 10×, oc. 12.5×)

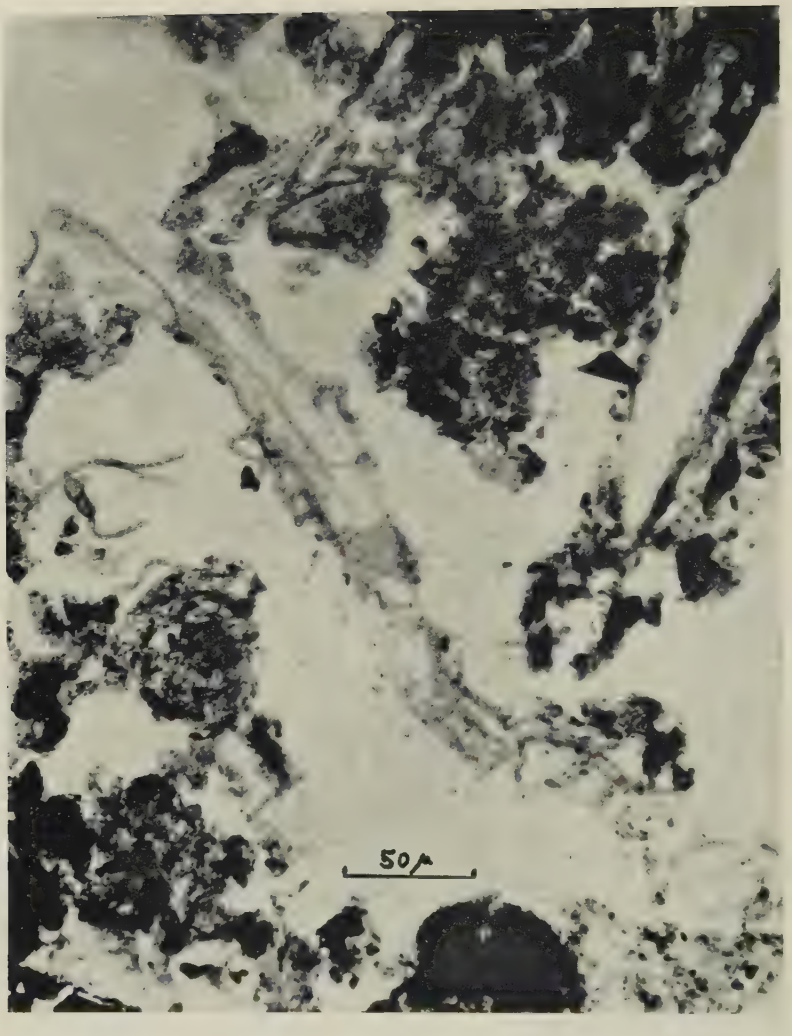

Plate IV. Very thin rootlet webbed in by hyphae. (objective 10×, oc. 12.5×)

they were not found to be as satisfactory as the quadruple staining of Johansen. Brilliant cresyl blue and Azur II give too heavily stained preparations.

Remarks

1. In winter during conditions of hard frost there is no point in placing the soil samples in the refrigerator at −10°C. When such samples thaw some of the animals rendered inactive by the cold become active again. Under normal conditions all animals are killed. Although attemps have been made, we have not yet succeeded in simultaneously killing and fixing the animals present in the soil samples in such a manner as to permit subsequent embedding in gelatin. It would be especially valuable if a method of fixing amoebae with outstretched pseudopodia could be found. For, with the present method, they invariably become spherical in shape before dying and thus become difficult to recognize.

2. Plastic blocks are made from sheet perspex 4.5 mm thick. The blocks measure 15 × 22 × 4.5 mm and have a hole 11.3 mm diameter in the centre. The diameter of this hole being the same as the maximum internal diameter of the steel sampler.

3. The gelatin will be liquefied in the hydrofluoric acid if the preliminary hardening process occupies considerably less than seven days. Sometimes even well hardened gelatin will be hydrolized in the hydrofluoric acid if the temperature rises much above 15°C.

4. Even after treatment for a period of three weeks in 50% hydrofluoric acid the nematodes appear to be well preserved and can be stained satisfactorily.

5. An attempt was made to use agar-agar and carboxy-methyl-cellulose (MZ. 501 and MZ. 851 manufactured by A.K.U.–Arnhem) in place of gelatin but the agar-agar liquefied in the hydro-fluoric acid and the specimens of C.M.C. were not suitable for the embedding process.

6. It seemed that, in order to show up the cavities remaining where the sand grains had been dissolved out, it might be more effective to use, for the second embedding, gelatin already stained in a contrasting colour instead of plain gelatin. However, attempts to do this using Chinese-ink failed.

7. Methylalcohol renders the gelatin cubes sufficiently firm so that they are very suitable for the cutting of thin sections. Methyl

alcohol also has an advantage over ethyl and propyl alcohol in that it ensures that the gelatin blocks retain their original dimensions and there is no shrinkage such as occurs so markedly when other alcohols are used. If, however, the blocks are stored in concentrated methyl alcohol for a long time, e.g. a month or so, the gelatin may show cavities.

SUMMARY

A new technique for study of small soil organisms in situ in unaltered soil is described.

The soil samples are cooled in a refrigerator at — 10°C to kill the animals. A small portion taken from a frozen soil sample, is slowly immersed in a solution of gelatin. When the specimen is infiltrated with gelatin and the whole cooled it is fixed in formalin to enable it to withstand treatment with hydro-fluoric acid for removal of sand grains. Subsequently the specimens are immersed in gelatin solution for a second time after which the specimens are affixed to wooden blocks which can be clamped in the microtome. Before sectioning, the embedded specimen affixed to the wooden block is hardened in methylalcohol after which it is possible to cut sections 7,5–10μ thick.

The most satisfactory staining procedure proved to be the quadruple staining method of Johansen. By this method nematodes, fungi, bacteria and amoebae are easily distinguishable from the soil particles.

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REFERENCES

1 Haarløv N., and Weis–Fogh, T., A microscopical technique for studying the undisturbed texture of soils. Oikos **4**, pp. 44–57, 1953.

2 Johansen, D. A., Plant Microtechnique. 523 pp. Mac-Graw-Hill, New-York and London, (1940).

3 Kubiëna, W., Micropedology. 318 pp. Collegiate Press Inc., Iowa (1938).

Plant and Soil VIII, no 1 August 1956

ON THE PHYSIOLOGY OF TUBER FORMATION IN POTATOES

I. PREMATURE TUBER FORMATION

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INTRODUCTION

The study of tuber formation in *Solanum tuberosum* L. is one of the most fascinating subjects in plant physiology. De Vries[14 15] and Vöchting[12] both did classical work on normal tuber formation in the potato. They showed that at a certain stage of plant growth the organic compounds produced by assimilation are not primarily used for further development of leaves, stalks and roots, but that an important part is used for tuber formation. According to the findings of De Vries an accumulation of nutrient substances, or an increase in the concentration of nutrient substances in the neighbourhood of a bud, is essential for tuber formation.

Besides normal tuber formation a number of types of abnormal tuber formation are known:

1. Tuber formation on the aerial part of the plant, which results when the transport of assimilates to the underground part is impeded, for instance, by ringing the stems, by removing daughter tubers, or by the attack of pathogens such as *Rhizoctonia solani*. The phenomenon is due to the accumulation of excess nutrient substances near buds on the stems (De Vries[14], Vöchting[12]).

2. Secondary tuber formation. Two types are known (Krijthe[4]): *a*) Secondary tuber formation in which only a few eyes of the tuber continue to grow and form fresh small tubers on top of the first one. *b*) Secondary tuber formation in which the following grow from the top of the tuber, or from one or two eyes close to the top: (i) stolons on which tubers develop, or (ii) stolons with tubers on the top and on the branch stolons of these stolons, or (iii) haulms, which form leaves above ground and one or two stolons bearing a tuber underground.

3. Premature tuber formation, during storage of tubers in the light, if storage lasts a long time and/or takes place at a high temperature (De Vries[15], Wellensiek[17]). According to Wellensiek[16 20] excess

transpiration or assimilation or possibly both may lead to an increase of nutrient compounds in the sprouts.

4. Premature tuber formation during storage of tubers in the dark may also occur when storage lasts a long time and/or takes place at a high temperature. In this case, also development of tubers is induced by an increase in the concentration of nutrient substances as a result of loss of moisture (De Vries[15], Wellensiek[16]).

5. Premature tuber formation induced by storing tubers in a desiccator over $CaCl_2$, which was obtained by Wellensiek[15] and explained by him in the same way.

6. Premature tuber formation on white sprouts formed in the dark on tubers which could take up water by means of a lampwick dipped in a flask of water, the other end being inserted in a small hole in the tuber (Van Schreven[9]). In this case tuber formation could not be due to loss of moisture or to assimilation.

7. Premature tuber formation on hair sprouts or spindle sprouts, described by Harvey *et al.*[1]. They found that tubers of the variety White Rose with hair sprout formation contained far more reducing sugars and total sugar than tubers with normal sprout formation.

8. Premature tuber formation in early planted potatoes. In this case daughter tubers are formed in the soil without development of green parts above ground. Investigators who have studied this type of tuber formation are Schacht[8], Vilmorin[10], De Vries[14], Vöchting[11 13], Wellensiek[16 17 18 19 20], Oortwijn Botjes[6 7], Krijthe[2 3], Van Schreven[9], Nijdam and Ros[5].

Though the problem of premature tuber formation may be considered as being largely solved in relation to practice, the real causes are still little understood.

As early as 1883 Vilmorin[10] had shown that premature tuber formation in the Marjolin variety was encouraged by desprouting the tubers before planting. Vöchting[13] working with the same variety found that pregerminated potatoes developed mainly small new tubers and not sprouts when the temperature after planting was maintained at 6–7°C.

Wellensiek[16 17] showed that a predisposition to premature tuber formation could be induced in the variety Duke of York (Eersteling) by storing the tubers at high temperatures in the dark and by desprouting frequently before planting. Premature tuber formation was ascribed by him to an abnormally high concentration of dissolved nutrient substances in the tubers resulting from loss of water due to sprout formation and desprouting during storage.

Oortwijn Botjes[6] planted tubers in moist sand and removed the sprouts as soon as they reached the soil surface. Tuber formation took place notwithstanding that the mother tubers had absorbed plenty of water through their roots. From his experiments he concluded that Wellensiek's hypothesis that premature tuber formation was due to "loss of water" is incorrect. Since sprouts contain two ot four times as much protein as the mother tuber, he supposed that desprouting causes a lack of proteins in the tuber and

thus an increase in the relative proportion of carbohydrates. He suggested that lack of protein in the tuber would cause poor sprout formation and that the feeble sprouts would not be able to assimilate the nitrogen-free organic substances.

When mother tubers were allowed to absorb water during storage by means of lampwicks inserted in small holes in the tubers the other ends dipping in flasks of water it was found by Van Schreven[9] that the uptake of water resulted in an increased weight of the mother tubers and in a greater weight of sprouts, but that the dry matter content of the sprouts was decreased. After desprouting and subsequent planting in sand the development of young tubers in the dark took place much earlier if the mother tubers had taken up water during storage than if they had been given normal storage without uptake of water. These experiments prove that premature tuber formation is not caused by a lack of water in the mother tuber as a result of sprout formation in the period of germination, as suggested by Wellensiek. Moreover it was found that the ratio of starch to proteins in the mother tuber decreased during storage and also during the growth period, in the latter case much faster than in the former. In other words, during storage and growth relatively more starch than protein was transformed. This can be explained by the fact that carbohydrates are used not only in the development of sprouts, roots and tubers, but also in respiration.

Nijdam and Ros[5] made studies to determine whether, before planting there is any differentiation in certain buds that would predetermine their development into stolons. At various periods sprouts that were allowed to develop in light were removed and the subsequent development of the remaining buds studied. The investigations failed to show that certain lateral buds in the eyes had undergone differentiation before planting such that their development into stolons was already determined. Nor did it appear that stolon formation was encouraged when, under conditions chosen by the investigators, tubers were compelled to use axes of higher order for sprout development.

With the exception of the studies of Nijdam and Ros, therefore, investigations on premature tuber formation have been directed to the physiological factors concerned.

In the present investigation premature tuber formation was artificially induced and a study was made of the factors favourable for the development of this type of tuber formation.

METHODS

The experiments were made with the early variety Duke of York (Eersteling). After different types of pre-treatment the tubers were grown in high glass tumblers containing ± 1600 g of a sandy loam soil. The tubers were planted at a depth of 10 cm. During the experiment the moisture content of the soil was maintained at ± 60 per cent of the water holding capacity by

regularly weighing the tumblers and replacing the water lost by evaporation.

The influence on premature tuber formation of (A) sprout formation during storage, (B) temperature after planting and (C) size of mother tubers was studied.

Tubers which had been stored till February 14th at 5°C and had not yet formed any sprouts were kept for a month in a dark cellar at 12°C and afterwards in a dark cupboard at ± 20°C. On May 10th these "pregerminated" tubers (series A) had formed many sprouts, they were also very much shrivelled and had lost a substantial amount of their reserve-materials.

A second series of tubers (series B), which had been stored till January in a cool store room for potatoes, was placed afterwards in a greenhouse at a relatively low temperature (± 10°C). On May 10th these tubers were firm to the touch and had developed small green sprouts (0.5 to 1 cm long). Evidently they had lost only a small amount of reserve-materials.

On May 10th the tubers of both series were desprouted and planted in the tumblers. From each series one group was placed in the dark at room temperature (groups A_1 and B_1) and another group in a refrigerator at 5°C (groups A_2 and B_2). Since the tubers in this refrigerator had not formed any roots or shoots on June 20th the temperature was raised to 7°C. On July 6th the temperature in the refrigerator was raised again, this time to 9°C, since practically no roots had formed yet.

RESULTS

On June 28th, in 6 of the 10 tumblers of group A_1 (20°C) shoots had formed and in 3 cases premature tuber formation occurred, but only when very small seed potatoes were used. On July 9th most shoots in group A_1 were already dead and dried up and on that day this group was harvested (Table I).

TABLE I

Influence of the weight of pregerminated and desprouted mother tubers on the formation of shoots above the soil and on premature tuber formation (temperature 20°C)

Number of tuber group A_1	Weight of tuber after desprouting, grams	Weight of sprouts formed during storage, grams	Development of shoots above the soil	Premature tuber formation
1	74.86	5.34	3 shoots	—
2	68.31	5.66	8 ,,	—
3	64.18	6.12	5 ,,	—
4	49.18	3.56	1 big shoot	—
5	37.47	4.97	no shoots	+
6	23.32	2.74	10 small shoots	—
7	20.24	2.00	no shoots	+
8	20.10	1.20	,, ,,	+
9	17.08	1.99	1 small shoot	—
10	16.27	1.95	no shoots	— *

* rotted, no young tubers formed.

Plate I. Premature tuber formation in the variety Duke of York. The mother tubers had been kept for 2 months in the dark at room temperature. Then they were desprouted and grown at 5-9° C.

Plate II. Premature tuber formation in the variety Duke of York, in some cases accompanied by secondary tuber formation. Both tubers at the left without premature tuberation. The mother tubers had not been pregerminated in the dark. They were planted on the same day as the tubers of Plate I and also grown at 5-9° C.

The tubers in series A_1 which did not develop shoots above the soil were all small ones. All tubers of group B_1 formed sprouts so that no cases of premature tuber formation were found in this series.

At the end of July only one of the 12 tubers of group A_2 (pregerminated in the dark, desprouted and grown at 5°–9°C) had formed feeble roots along the wall of the tumbler. At that time 10 of the 12 tubers of group B_2 had formed roots along the wall of the tumbler and 6 tubers had formed shoots. On August 14th 8 tubers of this group had formed shoots and the experiment was ended since the shoots began to decay.

Premature tuber formation occurred on all tubers in group A_2 and on 33 per cent in group B_2. The yield of shoots, roots and tubers and the percentages of premature tuber formation of the four groups are given in Table II.

TABLE II

Mean yield per tuber of shoots, premature tubers and roots in grams together with the total number of tubers and the percentage giving premature tuber formation per group

Treatment	Mean weight of desprouted mother tuber	Mean weight of shoots	Mean weight of young tubers	Mean weight of roots	Total number of young tubers per group	Cases of premature tuber formation
	g	g	g	g		%
Group						
A_1 (20°)	39.10	0.81 *	23.83	0.64	41	33
B_1 (20°)	18.26	19.11	3.86	2.15	20	0
A_2 (5°-7°-9°)	33.09	0.00	3.23	0.00	54	100
B_2 (5°-7°-9°)	17.25	4.32	1.16	0.86	12	33

* Most shoots dead and shrivelled at harvest time.

It is evident that pregermination of the mother tubers in the dark (group A_1) and a low temperature during growth (group B_2) both stimulated premature tuber formation. When both factors operate the conditions for premature tuber formation are most favourable (group A_2). Pregermination of the mother tubers at room temperature in the dark caused development of young tubers earlier, with feeble root and shoot production; a low temperature during growth caused a retardation of shoot and root formation.

As is shown in Plate I the mother tubers of group A_2, which had lost much water and reserve materials during storage and which

were grown at a low temperature, still had a shrivelled appearance at harvest time. These tubers had taken up practically no water, since root formation was very poor or absent.

The mother tubers of group B_2 (Plate II), however, which had not lost much reserve materials and water during storage and which were grown at a low temperature, had a normal appearance and felt firm to the touch at harvest time. In this group premature tuberation, in some cases, was accompanied with secondary tuber formation. According to Krijthe[4] the cause of secondary tuber formation may be sought in suspension of growth of tubers owing to unfavourable circumstances, in which drought plays the principle part. In the present investigation, however, drought could not play a role, as the moisture content of the soil was maintained at $\pm$ 60 per cent of the water holding capacity. Consequently under these conditions premature tuber formation could not have been caused by lack of water or organic matter.

SUMMARY

Experiments were carried out with tubers of the early variety Duke of York (Eersteling), some of which were stored at room-temperature in the dark and which had lost much of their reserve materials and water, while others, stored at a lower temperature, were still in good condition with short sprouts. Both lots were desprouted in May and planted in tumblers of sandy loam. Each lot was then divided into two groups, one being grown at room temperature in the dark and the other at 5°–9°C in a refrigerator.

Pregermination of the tubers in the dark and growth at a low temperature both favoured premature tuber formation. When the two factors operated together the conditions for premature tuber formation were most favorable. Pregermination in the dark and desprouting caused earlier development of young tubers, with feeble root and shoot production; low temperature caused a retardation of shoot and root formation. At room temperature premature tuber formation occurred only when the mother tubers were very small and when they had lost much reserve material during storage. It appeared that premature tuber formation was correlated with conditions which checked root and sprout formation after planting, but not necessarily with loss of reserve materials in the mother tuber.

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REFERENCES

1 Harvey, R. B., Reichenberg, A., Lehner, B. and Hamm, P. C., Hair sprout of potatoes. Plant Physiol. **13**, 186–193 (1944).

2 Krijthe, N., Over de invloed van temperatuur en licht tijdens de bewaring van pootaardappelen op de oogst van Eerstelingen. Lab. Plantenphysiol. Onderzoek. Wageningen, Mededeel. **73**, Verslag. Landbouwk. Onderzoek. **54**, 1–40 (1948).

3 Krijthe, N., Behandeling van pootaardappelen met veel spruitverlies ter voorkoming van onderzeeërvorming. Med. NAK **1**, 85–86 (1948).

4 Krijthe, N., Observations on the formation and growth of tubers on the potato plant. Netherl. J. Agr. Sci. **8**, 291–304 (1955).

5 Nijdam, F. E. and Ros, B., Ontijdige knolvorming bij de aardappel in verband met de morphologische toestand van de poter (Premature tuber formation in connection with the morphology of the tuber). Tijdschr. Plantenz. **59**, 256–259 (1953).

6 Oortwijn, Botjes, J. G., Ontijdige knolvorming bij aardappelen. Tijdschr. Plantenz. **33**, 1–13 (1927).

7 Oortwijn Botjes, J. G., Droge bewaring en onderzeeërvorming bij aardappelen. Tijdschr. Plantenz. **35**, 108–111 (1929).

8 Schacht, H., Bericht über die Kartoffelpflanze und deren Krankheiten. Berlin, 1856.

9 Schreven, D. A. van, Over ontijdige knolvorming bij vroege aardappels (Premature tuber formation in early potatoes). Tijdschr. Plantenz. **55**, 290–308 (1949).

10 Vilmorin, A., Les Plantes Potagères, p. 489. Paris, 1883.

11 Vöchting, H., Über die Bildung der Knollen. Bibliotheca Botanica **4** (1887).

12 Vöchting, H., Zur Physiologie der Knollengewächse. Jahrb. Wiss. Botan. **34**, 1–148 (1900).

13 Vöchting, H., Über die Keimung der Kartoffelknollen. Botan. Z. **60** (1902).

14 Vries, H. de, Beiträge zur speciellen Physiologie landwirtschaftlicher Kulturpflanzen IV. Keimungsgeschichte der Kartoffelknollen (Opera e periodicis collata **3**, 200: 246 (1878); Landwirtsch. Jahrb. **7**, 217–249 (1878).

15 Vries, H. de, Beiträge zur speciellen Physiologie landwirtschaftlicher Kulturpflanzen. V. Wachstumgeschichte der Kartoffelpflanzen. Landwirtsch. Jahrb. **7**, 591 (1878).

16 Wellensiek, S. J., Ontijdige knolvorming bij vroege aardappels. Mededeel. Landbouwhoogesch. Wageningen **27**, no. 3 (1923).

17 Wellensiek, S. J., Een onderzoek naar de factoren, die ontijdige knolvorming bij vroege aardappels bepalen. Thesis, Wageningen (1924); Tijdschr. Plantenz. **30**, 177–226 (1924).

18 Wellensiek, S. J., De invloed van luchtvochtigheid op pootaardappelen tijdens de bewaring. Tijdschr. Plantenz. **35**, 13–24 (1929).

19 Wellensiek, S. J., Een antwoord aan J. Oortwijn Botjes en een advies aan de practijk inzake droge of vochtige bewaring van aardappels. Tijdschr. Plantenz. **35**, 234–236 (1929).

20 Wellensiek, S. J., The physiology of tuber formation in *Solanum tuberosum* L. Mededeel. Landbouwhoogesch. Wageningen **33**, 6–42 (1929).

Plant and Soil VIII, no 1 August 1956

ON THE PHYSIOLOGY OF TUBER FORMATION IN POTATOES

II. INFLUENCE OF SOME ORGANIC COMPOUNDS ON TUBER AND SPROUT FORMATION OF POTATOES ASEPTICALLY GROWN IN THE DARK

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1. INTRODUCTION

As was shown by Vilmorin[26], Wellensiek[29 30] and Van Schreven[18], tuber formation in *Solanum tuberosum* L. takes place earlier when sprouts have been formed during storage and the tubers have been desprouted before planting. Wellensiek showed that premature tuber formation is promoted by storing the tubers in the dark at high temperature and by desprouting the tubers frequently before planting. Van Schreven[18] found that factors that are favourable for sprout formation during storage (darkness, a high temperature, the uptake of water during germination) promote earlier formation of tubers. Storing the tubers in the dark at room temperature and desprouting, resulting in a lack of reserve materials, caused a weak root and shoot growth after planting and this went hand in hand with earlier formation of tubers (Van Schreven[19]). It was also found that low temperatures during growth, which caused a retardation of shoot and root growth, stimulated premature tuber formation and it was concluded that this could not be due to lack of reserve materials in the mother tuber (Van Schreven[19]).

When sprouts are formed during storage in the dark not only water and inorganic substances, but also organic materials, such as sugars, proteins, auxins, auxin precursors and vitamins are withdrawn from the mother tuber. It was thought that it would be interesting to examine the effect of such organic materials on the sprout and tuber formation of potatoes grown in the dark.

2. EXPERIMENTAL METHODS

To investigate the influence of the uptake of organic substances by the mother tuber under aseptic conditions, the following technique was employed.

Each tuber was treated for 30 minutes in 0.1% solution of $HgCl_2$ and rinsed with sterile water. With a sterile borer a hole of 6.5 mm diameter was made three quarters of the way through the flesh of the tuber and into this hole the end of a cotton lampwick was inserted with a sterile pair or forceps. The lampwick ran through a glass-tube passing through the rubber stopper of a dispensing bottle and hung in a culture solution. The end of the tube, which protruded $\pm$ 1.5 cm above the stopper was stuck for a small distance into the hole in the tuber. In this way the tuber was fixed rather firmly upon the tube while the inner flesh (parenchyma and vascular tissue) was in contact with the lampwick which could absorb nutrient solution from the bottle. To avoid contamination the edge of the wound, which was still exposed to the air, was covered with a mercurial ointment. Sterile air was allowed to enter the bottle through a bent tube closed with a wad of cotton wool to replace the volume of solution taken up by the tuber. The bottles with 150 or 250 ml culture solution were sterilized for half an hour to one hour at 120°C. During sterilization the protruding part of the tube containing the lampwick was covered by a short rubber tube (closed at one end with a clip) to prevent contamination of the lampwick after sterilization. Shortly before placing the tuber upon the bottle this rubber tube was removed. As a further precaution, the top end of the tube with the protruding lampwick was slightly flamed before inserting the lampwick and top end of the tube into the hole in the tuber.

By using this procedure practically no contamination of the tubers or solution cultures took place. After several weeks tubers allowed to germinate in this way in the dark developed young tubers on the sprouts. These tubers were counted at intervals and the fresh mother tubers were weighed individually before starting the experiments. To compensate for the differing weights of the mother tubers the yields of young tubers and sprouts of each series were expressed as percentages of the weights of the mother tubers. The influence of the different organic materials was studied both on tubers which had not yet formed sprouts and on desprouted tubers which had been kept for several weeks at room temperature in the dark before the experiment began. For the sake of brevity the first type of tubers are called "non-pregerminated tubers" and the second type "pregerminated tubers". In these experiments the early variety Eersteling (Duke of York) was used.

3. ANALYTICAL METHODS

A. *Sugars*

For determining reducing and total sugar in the sap of mother tubers and sprouts, from 7 to 10 or more tubers were minced separately, the pulp squeezed out with a fruit press and the sap then centrifuged.

For the estimation of reducing sugar the picric acid method of Peacock and Brunstetter[17] was used, but in place of cylinders of potato tissue 0.2 ml expressed sap was used. For the determination of total sugar 10 ml sap was treated with a neutral solution of lead acetate. After filtration the lead was precipitated with *N*a-oxalate and the solution filtered again into a 50-ml flask. After addition of 2 ml 6.2 *N* HCl, distilled water was added up to 50 ml and the solution kept for 24 hours at room temperature. One ml of this solution, corresponding to 0.2 ml sap, was neutralized with 1 *N* NaOH. The total content of reducing sugar and converted sucrose was then determined according to the picric acid test, comparing it with a standard series with increasing amounts of glucose.

B. *Starch*

The relative amounts of starch in the tissues were indicated with a diluted solution of potassium iodide containing iodine.

C. *Protein and soluble non-protein nitrogen*

Protein and soluble non-protein nitrogen were determined according to the method of Mulder[12] (p. 64) in 10 g of the average samples from 7 to 10 or more tubers, which were ground in a porcelain mortar.

4. Influence of (1) pregerminating, (2) pregerminating and desprouting and (3) storage of the mother tubers at 2°C, without addition of organic substances

Four series of tubers from the same lot of potatoes were treated as follows. One series (series 1) was kept in the light during day time in a rather cool place (10–15°C). A second sereis (series 2) was kept in the dark for 7 weeks (from 22 December 1949 to 9 February 1950 at a temperature of 2°C. A third series (series 3) was placed for 2 months in the dark at room temperature. A fourth series (series 4) was treated as series 3, but at the end of the "germination period", on 9 February, the sprouts were removed.

On 9 February, 10 tubers of each series were placed on dispensing bottles containing sterile water. The first young tubers appeared after ± 8–9 weeks in the series 3 and 4 (Fig. 1, A). In series 2 the first tubers appeared after 15 weeks and in series 1 after 20 weeks. Evidently the pregermination of the mother tubers induced earlier formation of young tubers. As regards the time at which tuber formation began it made no difference whether the pregerminated tubers had been desprouted or not. In series 2 tubers were formed 5 weeks earlier than in series 1. It is clear, therefore, that this earlier tuber formation resulted from the storage of the mother tubers at

2°C for 7 weeks. It is known that tubers which have been kept for several weeks at 2°C contain much more sugars than tubers kept at higher temperatures. Possibly this higher sugar content has stimulated earlier tuber formation in series 2.

From the series 1, 3 and 4 sprouts and young tubers were harvested

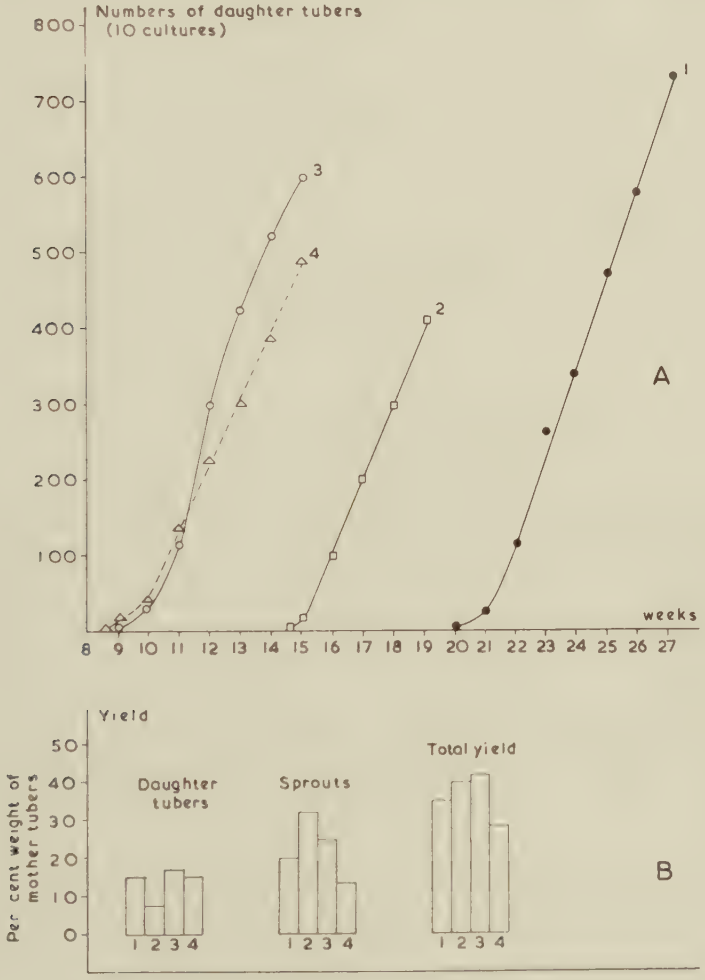


Fig. 1. *1*) Tubers not pregerminated; *2*) tubers stored for 7 weeks at 2°; *3*) tubers pregerminated for 2 months at room temperature in the dark, not desprouted; *4*) as *3*, but desprouted. The tubers of the four series had been placed on bottles with sterile water.

seven weeks after the formation of the first tubers; from series 2 they were taken five weeks after the development of the first tubers, when the first symptoms of dying off of the sprouts were noticed. As can be seen from the yields plotted in Fig. 1B series 2 gave the smallest yield of young tubers, but the greatest weight of sprouts. Series 1 produced more tubers than series 3 and 4, but most tubers in series 1 were smaller than in the latter series.

In another experiment potatoes were kept in the dark at 2°C from 13 December to 9 January and grown afterwards on water (series A). Another lot of potatoes was kept in the dark at room temperature from 7 December to 9 January and desprouted before growing the tubers on water (series B).

On 29 March a number of the "plants" of series B had developed small young tubers. As in the former experiment the formation of young tubers occurred later when no sprouts had developed during germination, since tuber formation in series A began in the middle of May.

Ten "plants" of each series were analysed on several dates during growth on the bottles and the chemical data are recorded in Table I.

At the start of the experiment the tubers of the series stored at 2°C contained much more reducing sugar and sucrose than those stored at room temperature. The content of reducing sugar and total sugar of the mother tubers in series A decreased first and increased again at a later growth stage. In the tubers of series B these contents increased gradually till 29 May. In both series the content of total sugar of the sap was lower on 17 June as compared with that on 29 May due to the decreased sucrose content.

During the growth of the plants the content of soluble non-protein nitrogen in the sap of the mother tuber decreased and this took place more rapidly in the mother tubers of series B than in those of series A.

For the sprouts the following facts were found. During growth the content of protein nitrogen of the dry matter decreased gradually in both series. The soluble non-protein nitrogen of the sap of the non-desprouted series was somewhat higher than that of the desprouted series during the growth period on the bottles. As compared with the sprouts which had been removed during the germination in the dark there was first an increase in the content of reducing and total sugar followed by a gradual decrease of these contents.

TABLE I

(A) Effect of storing tubers at low temperature and (B) of pregerminating potatoes for 7 weeks in the dark at room temperature and desprouting them afterwards, on the contents of protein nitrogen, soluble non-protein nitrogen, total nitrogen, reducing sugar and total sugar and on the ratio of reducing and total sugar to soluble non-protein nitrogen during the development on water

Treatment		% of dry matter				% of sap			Ratio red. sugar / sol. non-protein N	Ratio total sugar / sol. non-protein N
		Protein N	Solub. non-protein N	Reducing sugar	Total sugar	Solub. non-protein N	Reducing sugar	Total sugar		
9 January										
Mother tubers	(series A)	0.95	1.03	10.66	18.65	0.19	2.00	3.50	10.5	18.4
,, ,,	(series B)	0.81	0.88	2.78	5.56	0.24	0.75	1.00	3.1	4.2
Sprouts *	(series B)	1.87	2.13	18.33	20.17	0.29	2.50	2.75	8.6	9.4
29 March †										
Mother tubers	(series A)	0.53	1.17	6.59	14.83	0.18	1.00	2.25	5.6	12.5
,, ,,	(series B)	0.48	1.06	11.48	18.90	0.15	1.70	2.80	11.3	18.6
Sprouts	(series A)	1.52	1.90	23.69	28.44	0.28	2.75	3.00	9.7	10.7
,,	(series B)	1.54	1.99	22.02	24.02	0.24	2.75	3.00	11.5	12.5
29 May										
Mother tubers	(series A)	0.49	1.28	18.06	22.66	0.15	2.10	2.63	14.0	17.5
,, ,,	(series B)	0.45	1.49	34.81	50.82	0.10	2.50	3.65	25.0	36.5
Sprouts	(series A)	1.36	1.86	15.33	17.03	0.27	2.25	2.50	8.3	9.2
,,	(series B)	1.43	1.86	11.51	19.43	0.25	1.60	2.70	6.4	10.8
17 June										
Mother tubers	(series A)	0.40	1.34	25.24	27.98	0.12	2.25	2.50	18.8	20.8
,, ,,	(series B)	0.36	1.38	29.13	31.47	0.12	2.50	2.70	20.8	22.5
Sprouts	(series A)	0.94	2.24	10.79	11.35	0.35	1.70	1.80	4.8	5.1
,,	(series B)	1.15	1.79	9.44	11.34	0.28	1.50	1.80	5.4	6.4

* These sprouts had been removed before placing the tubers on the bottles.

† On this date a number of the plants of series B had developed small young tubers.

In the mother tubers the ratio of reducing sugar to soluble non-protein nitrogen and also the ratio of total sugar to soluble non-protein nitrogen was at the start lower in series B than in series A but this situation soon became reverted and during the growth period this ratio remained higher in series B than in series A, most strikingly on 29 May. In the sprouts the highest ratio of soluble carbohydrates to soluble non-protein nitrogen was found on 29 March in series B. On this date a number of the plants of this series

had developed the first young tubers. During the further development of the young tubers the proportion of soluble carbohydrates to soluble non-protein nitrogen decreased gradually. The earlier tuber formation in the pregerminated and desprouted series is correlated with a higher proportion of the soluble carbohydrates to soluble non-protein nitrogen during the growth of the plants in this series as compared with the other series.

5. INFLUENCE OF SUGARS

It has been shown by several investigators, e.g. by Müller-Thurgau [14] [15], Appleman [2] [3], Peacock and Brunstetter [17], Wright [31], Denny and Thornton [6] [7], Alexander *et al.* [1] and Terman [24], that during storage at low temperatures (0°C–10°C) saccharose and reducing sugars (glucose and levulose) accumulate in potato tubers, due to hydrolysis of starch.

In all 19 varieties studied by Denny and Thornton [7] the content of reducing sugar during storage at 5°C rose to about 3.0 mg per ml sap and in some varieties to 10 or even more than 20 mg per ml sap. Much lower contents were found after storage at 7° or 8°C. Appleman and Smith [4] found that potato tubers accumulated sugar for a time at 30°C as well as at temperatures just above freezing. Watada and Kunkel [28] showed that the accumulation of reducing sugar in cold storage varied greatly between different varieties: 13 varieties contained more than 20 per cent of their dry matter as reducing sugar, whereas 4 varieties contained less than 6 per cent of their dry matter as reducing sugar when stored at 33°F for 14 weeks. Varieties differ greatly in ability to change reducing sugar back to starch after it has accumulated.

Experimental section

Tubers which had been stored for 7 weeks at 2°C in the dark and tubers which had been kept for 2 months at room temperature in the dark and had been desprouted afterwards, were placed on bottles containing water, 1.8% glucose or 3% glucose, respectively (series 1 to 6 in Fig. 2). Since fresh tubers may contain up to 5.23 per cent reducing sugar and up to 7.28 per cent total sugar when keeping them at 0°C (Wright [31]) and since it was found in the former experiment that the content of total sugar in the sap of the variety used rose to 3.65 per cent during the growth on water, a concentration of 1.8 or 3.0% glucose in the bottles was not considered to be harmful. Presumably this was not so, for the uptake of water by the tubers was

checked in presence of both concentrations of glucose (Plate I–IV).

Tubers of the desprouted pregerminated series kept their shrivelled appearance on the glucose solutions in contrast to the tubers placed on pure water which lost this appearance by uptake of water (Plate II). Tubers of the series kept for 7 weeks at 2°C, which were firm to the touch at the start of the experiment and contained 3.5% total sugar, gradually got a shrivelled appearance on the solutions containing glucose (Plate I). Evidently also for these tubers concentrations of 1.8 and 3.0% glucose were too high. This may be declared by the already long known fact (Müller-Thurgau[14]) that a high sugar content, found in potatoes after storage at low temperatures, decreases rapidly (within ± 10 days) after exposing the tubers to a temperature of ± 20°C.

The tubers and sprouts of the glucose series contained much starch at the end of the experiment in contrast to the tubers and sprouts of the water series as could be shown with the iodine test (Plates V and VI). At that time the central part of the tubers of the water-series was free from starch and only a zone beneath the skin contained starch. This zone was smaller at the stem-end than at the top-end of the tuber and generally the zone was broader in the mother tubers kept for 7 weeks at 2°C than in those of the pregerminated series. Most tubers of the glucose series contained also starch in the central part. Evidently the reaction starch → sugar was checked by the presence of glucose in the solution. It is interesting to note that although the sprouts in the glucose-series contained decidedly more starch than those of the water cultures, the development of young tubers was markedly delayed in presence of glucose.

After 15 weeks, cylinders of about 0.5 cm diameter were taken transversely from the tubers of each series with a corkborer. The middle part and the end parts of the borings were analysed separately. It could be shown that the end parts contained distinctly less reducing sugar than the central part. Borings of tubers originally pregerminated in the dark, which had been grown on water, contained more reducing sugar than corresponding tubers grown on 1.8% glucose and the latter contained more reducing sugar than those grown on 3% glucose (2.7, 2.0 and 1.6 per cent, respectively). However, the sprouts of the tubers, which had been grown on water contained less reducing sugar than those grown on 1.8 and 3% glucose (1.5, 1.9 and 1.8 per cent, respectively).

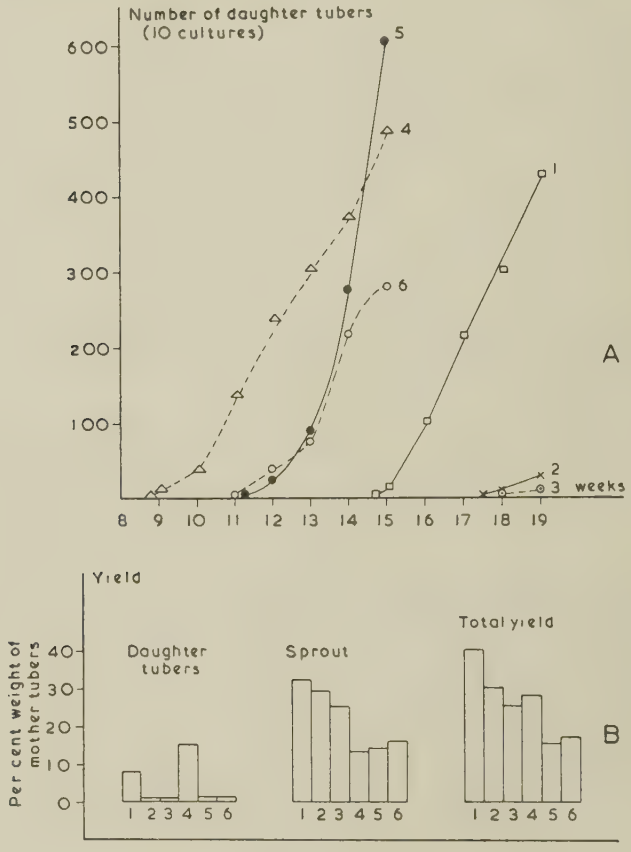


Fig. 2. *1*) Tubers kept for 7 weeks at 2°C, grown on water (250 ml); *2*) as *1* + 1.8% gluocse; *3*) as *1* + 3% glucose; *4*) tubers pregerminated for 2 months in the dark, desprouted, grown on water; *5*) as *4* + 1.8% glucose; *6*) as *4* + 3.0% glucose.

Plate I
Non-pregerminated tubers grown for 28 days in the dark. From left to right: on water, on a 1.8% glucose solution, and on a 3% glucose solution. The tubers grown on the glucose solutions have a shrivelled appearance.
Plate II
Tubers kept for 2 months in the dark at room temperature, desprouted, grown for 28 days. From left to right: on water, on a 1.8% glucose solution, and on a 3% glucose solution. The tuber placed on water has lost its shrivelled appearance.
Plate III
Non-pregerminated tubers grown for 16 weeks in the dark. From left to right: on water, on a 1.8% glucose solution, and on a 3% glucose solution. On the glucose solutions the formation of young tubers has been delayed and checked.

Plate I

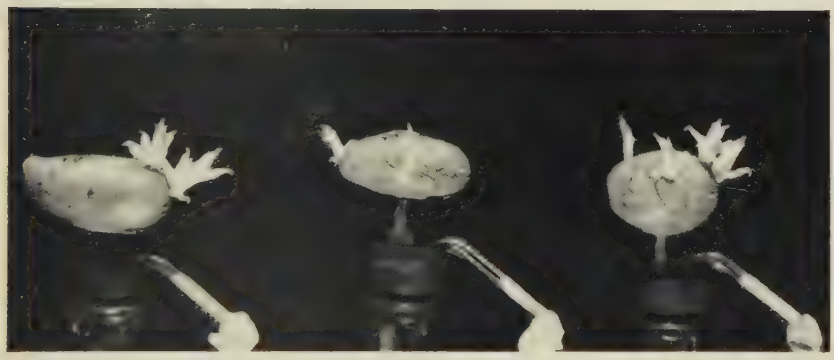


Plate II

Plate III

Plate IV

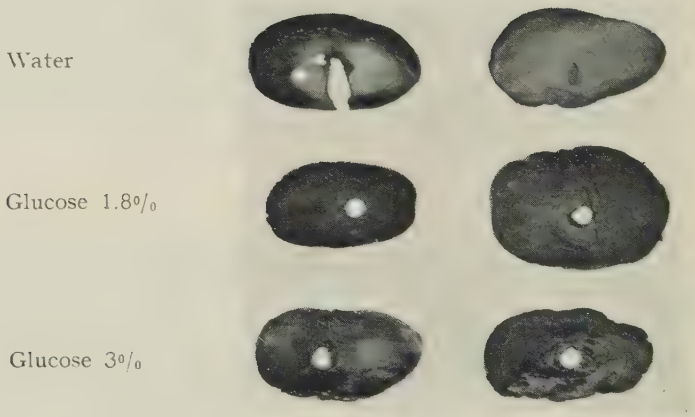


Plate V Not desprouted Desprouted

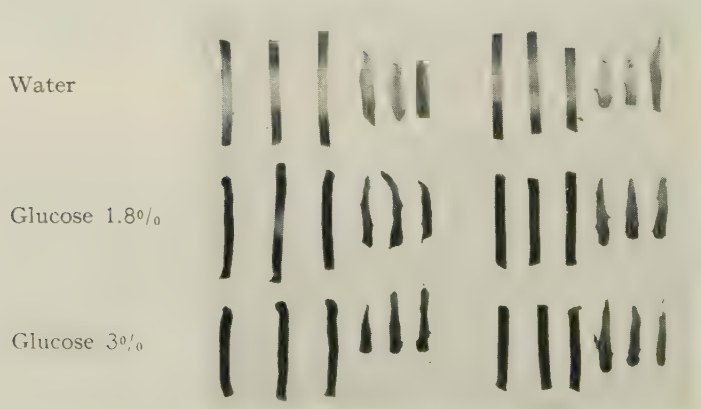


Plate VI Desprouted Not desprouted

As can be seen in Fig. 2A the development of young tubers was delayed for 2 to 3 weeks when the solution contained glucose. The development of sprouts was somewhat checked by the glucose when the mother tubers had not formed sprouts before (series 2 and 3), but a little stimulated when the mother tubers had been pregerminated and desprouted afterwards (series 5 and 6). The yield of tubers was greatly reduced in the series with glucose, more in the series 2 and 3 than in the series 5 and 6 (Figure 2B). In the presence of glucose most young tubers were very small.

In another experiment the combined effect of glucose and saccharose was studied using a solution of 0.5% glucose and 0.5% saccharose. Mother tubers were used which had been pregerminated for 12 weeks at room temperature in the dark and which had been desprouted afterwards. In Fig. 3 the effect of the combination of both sugars on the formation and yield of young tubers is shown. This combination has also delayed and reduced the development of young tubers, but a somewhat greater yield of tubers was obtained as compared with 1.8% glucose (Fig. 3, and Plate VII).

Although the yield of the sprouts was not reduced by the sugar solutions, the sprouts were shorter than those from the water cultures and had a bushy appearance (Plate IV). When daughter tubers of the different cultures were kept at room temperature in the dark, those from the desprouted series grown on water formed shorter sprouts than those from the water culture series with tubers which were kept for seven weeks at 2°C (Plate VIII). As can be seen on this plate sprout formation was markedly checked in the young tubers formed on glucose + saccharose solution.

The influence of increasing amounts of saccharose (0, 1.0, 1.5 and 2 per cent) is shown on Plate IX. The mother tubers had been kept in the dark for two months and had been desprouted before being

Plate IV
Tubers pregerminated for 2 months and desprouted, grown for 16 weeks. From left to right: on water, on a 1.8% glucose solution, on a 3% glucose solution, and on a solution containing 0.5% glucose and 0.5% saccharose.
Plate V
Slices of non-pregerminated and of pregerminated and desprouted tubers which had been grown on different solutions, treated with an iodine-containing solution of potassium iodide.
Plate VI
Cylinders of potato flesh and slices of sprouts of pregerminated and desprouted tubers and of non-pregerminated tubers which had been grown for 15 weeks on different solutions, treated with an iodine-containing solution of potassium iodide. The cylinders and the sprouts of the glucose-series contained more starch than those of the cultures on water.

placed upon the solutions. At this time all tubers showed a shrivelled appearance as the tubers had lost much water during storage. The photograph was made 7 weeks later. The shrivelled condition of the

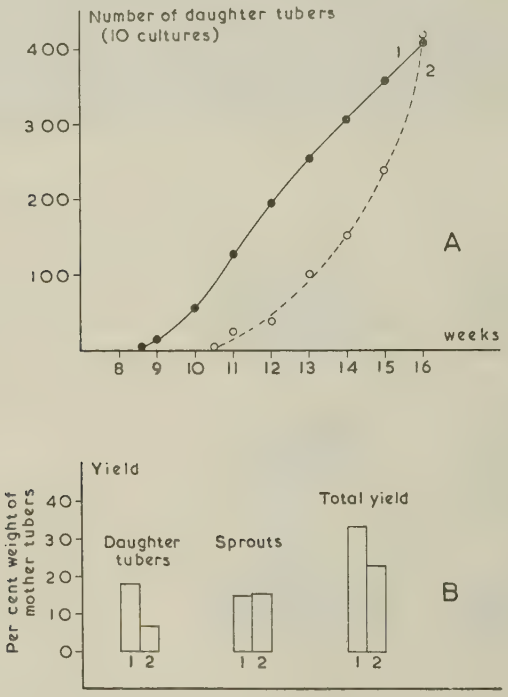
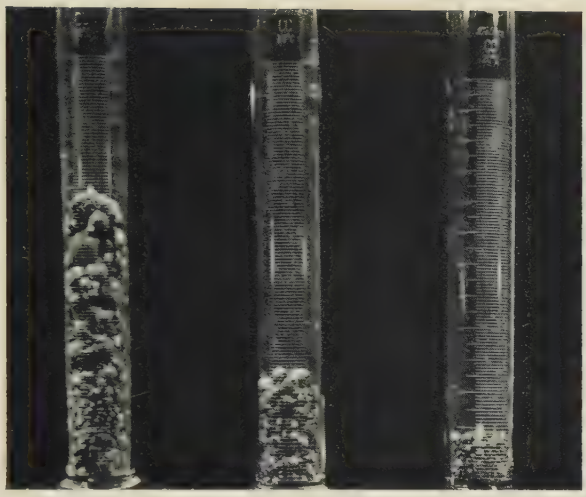


Fig. 3. *1*) Tubers pregerminated for 11 weeks in the dark, desprouted, grown on water (250 ml); *2*) as *1* + 0.5% glucose + 0.5% saccharose.

tuber placed on water has disappeared; the tubers placed on sugar solutions on the other hand, remained shrivelled and to an increasing degree as the concentrations of sugar increased. Also, the sprouts were shorter on tubers from the bottles with higher concentrations of sugar.

Plate VII
Yield of daughter tubers formed in cultures with pregerminated and desprouted tubers grown (*a*) on water, (*b*) on a solution of 0.5% glucose and 0.5% saccharose and (*c*) on a 1.8% glucose solution. Each series comprised the yield of 10 plants.
Plate VIII
Development of sprouts on daughter tubers formed (*a*) in cultures with pregerminated and desprouted tubers grown on water, (*b*) in cultures with non-pregerminated tubers grown on water and (*c*) in cultures with pregerminated and desprouted tubers grown on a solution containing 0.5% glucose and 0.5% saccharose.



a b c

Plate VII

a b

Plate VIII c

Plate IX

Plate X

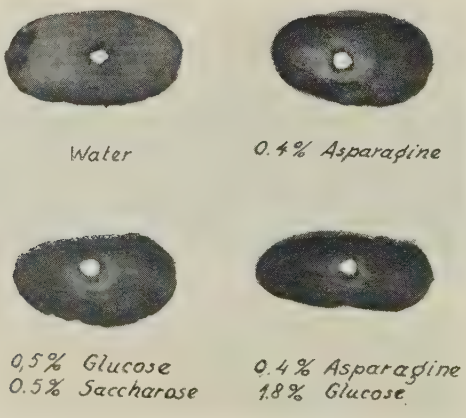


Plate XI

6. INFLUENCE OF NITROGENOUS ORGANIC COMPOUNDS WITH AND WITHOUT GLUCOSE

In the potato tuber the protein provides the building material for the protoplasm. It acts as reserve-material for the development of sprouts. An extensive investigation of the composition of the protein of the potato tuber has been carried out by Sjollema and Rinkes[20]. They found that it is built up of the same components normally present in most plant proteins so that it may be considered of the same value as other vegetable proteins. Using the method of Sjollema and Rinkes for the separation of protein from potato tubers Kiesel *et al.*[11] obtained two fractions, occurring in a ratio of 80 : 33. The smallest fraction proved to be soluble in water. As far as they were tested, both fractions contained the same nitrogenous compounds and the same types of amino-acids (histidine, arginine, tyrosine and proline) in the same proportion. Groot *et al.*[9] and Hofstee[10] also identified two fractions, the so-called tuberin (insoluble in water after dialysis) and the tuberinin (soluble in water after dialysis).

Determinations of the amino acid content of tuberin were carried out by Sjollema and Rinkes[20], by Groot[8] and by Slack[21]. After the introduction of the paper-chromatographic method a more detailed composition of the potato protein was given by Steward and Thompson[23 25] and by Mulder and Bakema[13]. Mulder and Bakema who investigated the effect of the mineral nutrition of potato plants on the amino-acid composition of the protein and the soluble fractions of the tubers, have given a critical survey of the amino-acid composition of potato tuber protein as obtained by various authors.

Neuberger and Sanger[16] examined the nitrogen distribution in ten varieties of the potato tuber. The relative proportions of insoluble N varied between about 8 and 12.5% of the total N. In the soluble fraction the relative proportions of protein varied approximately between 25 and 50%. Samples having a high relative protein content were low in amides (asparagine and glutamine) and *vice versa* so that the sum of protein and amides was fairly constant, *i.e.* about 70%. N due to α-amino-acids other than amides was found in only small amounts; it varied between 6 and 12%. Analysis made by Chick and Cutting[5] showed that the nitrogen in tubers of the

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Plate IX
Cultures of pregerminated and desprouted tubers grown for 49 days on solutions without and with increasing amounts of saccharose. From left to right: 0, 1.0, 1.5 and 2.0% saccharose.
Plate X
Cultures of pregerminated and desprouted tubers. From left to right: grown on water, on a 0.4% asparagine solution, on a solution of 0.4% asparagine and 1.8% glucose, and on a solution of 0.75% asparagine, 0.5% tyrosine and 0.1% glutamic acid.
Plate XI
Slices of pregerminated and desprouted tubers which had been grown on different solutions. The slices had been treated with an iodine-containing solution of potassium iodide.

King Edward variety was distributed as follows: true protein 39, amide 22, amino-acid 10 and basic N 25 per cent.

Steward and Street[22] isolated crystalline products from potato tubers in pure form, viz. 0.116 g tyrosine, 1.42 g asparagine and 1.51 g glutamine per 1000 g of fresh tubers. They considered glutamine as an active molecule more readily capable than other substances of donating nitrogen to molecules about to be synthesized into protein.

Experimental section

Tubers which had been pregerminated at room temperature in the dark for 12 weeks and which had been desprouted afterwards were placed on sterile solutions containing 0.75% asparagine, 0.5% tyrosine and 0.1% glutamic acid. Another series was placed on pure water. The effect of the nitrogenous compounds is seen in Fig. 4.

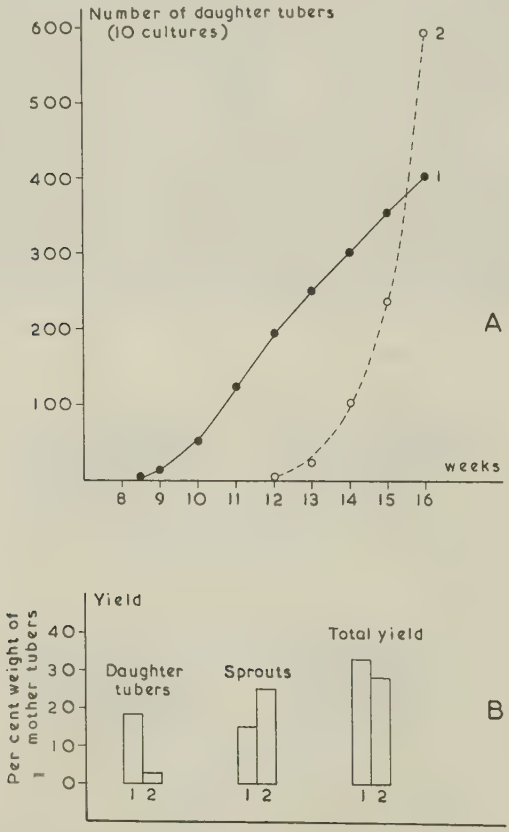

Fig. 4. *1*) Tubers pregerminated for 11 weeks, desprouted, grown on water (250 ml); *2*) as *1* + 0.75% asparagine, 0.5% tyrosine and 0.1% glumatic acid.

Although a greater number of young tubers was formed in presence of the nitrogenous materials the yield was greatly reduced and the development of young tubers was delayed for 3 weeks. The yield of sprouts, however, was greatly increased.

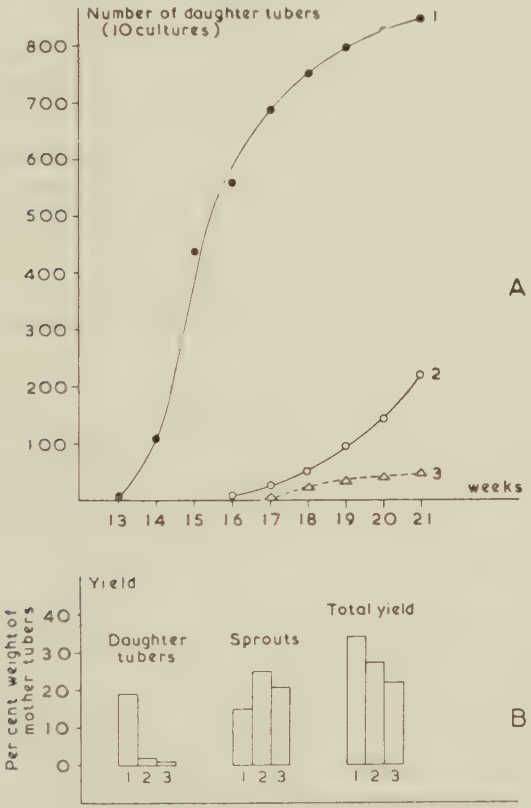

Fig. 5. *1*) Tubers pregerminated for 7 weeks, desprouted, grown on water (250 ml); *2*) as *1* + 0.4% asparagine; *3*) as *1* + 0.4% asparagine + 1.8% glucose.

In another experiment tubers which had been pregerminated at room temperature in the dark for 7 weeks and which had been desprouted afterwards, were placed on sterile solutions containing 0.4% asparagine or a combination of 0.4% asparagine and 1.8% glucose. As can be seen in Fig. 5A tuber formation was retarded by the asparagine for ± 3 weeks and still more delayed by the combina-

tion of asparagine and glucose. The yield of young tubers was greatly reduced by asparagine and a little more by the combination of asparagine and glucose (Fig. 5B). The yield of sprouts, however, was greatly raised by asparagine. A somewhat smaller increase of the sprouts was obtained by the combination of asparagine and glucose.

In presence of the nitrogenous compound and in presence of asparagine and glucose the sprouts showed a bushy appearance (Plate X). At the end of the experiment the mother tubers grown on asparagine contained more starch than those grown on pure water, as could be shown with the iodine test. The conversion of starch into sugar was checked even more, however, in presence of 0.5% glucose + 0.5% saccharose or in presence of 0.4% asparagine and 1.8% glucose (Plate XI).

After 21 weeks the mother tubers and sprouts of the series represented in Figure 5 were analysed; the results are shown in Table II.

TABLE II

Influence of nitrogenous organic compounds on the chemical composition of mother tubers and sprouts. Before placing the tubers on the bottles they had been stored in the dark for 7 weeks at room temperature and had been desprouted

Grown on	Tubers or sprouts	Moisture content %	% of dry matter		% of sap				Ratio reducing sugar / soluble non-prot. N	Ratio total sugar / soluble non protein N
			Protein N	Soluble non-protein N	Soluble non protein N	Red. sugar	Saccharose	Total sugar		
Water	tuber	91.0	0.71	0.83	0.08	2.80	0.70	3.50	35.00	43.75
	sprouts	85.0	1.17	1.53	0.27	1.70	1.05	2.75	6.29	10.18
0.4% asparagine	tuber	77.4	0.50	1.31	0.38	2.00	4.00	6.00	5.26	13.15
	sprouts	83.6	1.11	2.34	0.46	2.00	1.00	3.00	4.34	6.52
0.4% asparagine	tuber	79.2	0.43	1.35	0.49	1.40	3.60	5.00	2.83	10.20
and 1.8% glucose	sprouts	82.9	1.66	2.54	0.56	1.50	1.10	2.60	2.68	4.64

The content of soluble non-protein nitrogen in the tubers and sprouts was raised in presence of asparagine and glucose, as well as in presence of asparagine alone but most of all in the former case. The saccharose content of the tubers was also raised in presence of asparagine and to a somewhat lesser degree in presence of asparagine and glucose. The ratio of reducing sugar to soluble non-protein N and the ratio of total sugar to soluble non-protein N in the tubers and sprouts was greater in the water culture than in the other two cul-

tures. This was much more pronounced in the tubers than in the sprouts. It is evident, however, that a poor and retarded formation of young tubers is correlated with a low proportion of soluble carbohydrates to soluble non-protein nitrogen.

7. DISCUSSION

As found in earlier work it is clearly shown in these experiments that tuber formation is accelerated when sprouts have been formed during storage. As to the time of formation of the first tubers it does not matter much whether the pregerminated tubers have been desprouted or not, before placing them upon the bottles (Figure 1A). In fact, pregerminating at room temperature in the dark may be considered as the first phase of plant growth, attended by important metabolic changes. It is, therefore, self-evident that the formation of daughter tubers takes place at an earlier time the longer the mother tubers have been pregerminated. In the cultures with pregerminated tubers the time of formation of the first daughter tubers could be delayed by adding sugars or nitrogenous organic compounds. In presence of sugars the check of growth could be attributed to the impeded absorption of water. The sugar content of the tubers did not increase in presence of sugar without the addition of nitrogenous organic compounds, and the conversion of starch in the tuber was hindered.

Early tuber formation is correlated with a high proportion of soluble carbohydrates to soluble non-protein nitrogen in the tubers and in the sprouts. In earlier work [18] it was found that during growth in the dark relatively more starch than protein is transformed. In the present investigation it was shown that the proportion of soluble carbohydrates to soluble non-protein nitrogen in pregerminated tubers grown on water increases considerably during the first months of growth. In the sprouts this proportion had the highest value at the time of the development of the first tubers (Table I). When non-pregerminated tubers are used the highest proportion of soluble carbohydrates to soluble nitrogen in the tubers and in the sprouts is probably reached at a later moment and is correlated with a later development of young tubers. When nitrogenous organic compounds are taken up by the plant the ratio of soluble carbohydrates to soluble non-protein nitrogen is lowered (Table II). This is attended with a later development of young tubers.

Since tubers produce more sugars at a low temperature, it is most probable that the highest proportion of soluble carbohydrates to soluble nitrogen in the tubers and sprouts is reached at an earlier time when the temperature is kept low during the first development of the plant. This is attended with premature tuber formation. Evidently tuber formation can take place at a relatively low temperature (Vöchting [27], Van Schreven [19]). The growth of the sprouts in the soil will be influenced unfavourably by the development of the young tubers and also by the loss of nutrients when the tubers have been pregerminated and desprouted before planting. When moreover sprout formation is checked by the low temperature, so that the sprouts do not reach the soil surface, then premature tuber formation will be the result.

8. SUMMARY

Under aseptic conditions pregerminated and non-pregerminated tubers of the variety Duke of York were allowed to germinate in the dark and to take up pure water or solutions of organic substances. Under these conditions daughter tubers are formed on the sprouts.

Formation of young tubers occurs sooner when the tubers have been pregerminated beforehand in the dark at room temperature. The earlier tuber formation is correlated with a higher proportion of the soluble carbohydrates to soluble non-protein nitrogen. The highest proportion was reached earlier in the cultures with the pregerminated tubers and this was related to the first development of daughter tubers.

The formation of young tubers was delayed markedly in presence of sugars in the solution. This may be attributed to the impeded absorption of water and to a slower conversion of starch to soluble carbohydrates in the mother tubers.

In presence of nitrogenous organic compounds the formation of young tubers was also delayed considerably and in the mother tubers the conversion of starch took also place at a slower rate. The poor and retarded formation of young tubers is correlated with a low proportion of soluble carbohydrates to soluble non-protein nitrogen in the tubers and in the sprouts.

The results are discussed in connection with the problem of premature tuber formation.

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REFERENCES

1 Alexander, L. M., Schopmeyer, G. E. and Anderson, R. B., Potato storage and quality of french fries. I. Katahdin. Am. Potato J. **26**, 439–445 (1949).

2 Appleman, C. O., Physiological behavior of enzymes and carbohydrate transformations in after-ripening of the potato tuber. Botan. Gaz. **52**, 306–315 (1911).

3 Appleman, C. O., Biochemical and physiological study of the rest period in the tubers of *Solanum tuberosum*. Botan. Gaz. **61**, 265–294 (1916).

4 Appleman, C. O. and Smith, C. L., Effect of previous cold storage on the respiration of vegetables at higher temperatures. J. Agr. Research **53**, 557–580 (1936).

5 Chick, H. and Cutting, E. M., Nutritive value of the nitrogenous substances in the potato as measured by their capacity to support growth in young rats. The Lancet **2**, 667–669 (1943).

6 Denny, F. E. and Thornton, N. C., Factors for color in the producing of potato chips. Contr. Boyce Thompson Inst. **11**, 291–303 (1940).

7 Denny, F. E. and Thornton, N. C., The third year's results on storage of potato tubers in relation to sugar content and color of potato chips. Contr. Boyce Thompson Inst. **12**, 405–429 (1942).

8 Groot, E. H., Investigations into the biologically important amino acids in potato protein, in connection with its nutritive value. I–V. Arch. Néerl. Physiol. **28**, 277–361 (1947).

9 Groot, E. H., Jansen, L. W., Kentie, A., Oosterhuis, H. K. and Trap, H. J. L., A new protein in potatoes. Biochem. Biophys. Acta **1**, 410–414 (1947).

10 Hofstee, J., De oplosbaarheid van het aardappelglobuline (tuberine). Thesis, Groningen (1949).

11 Kiesel, A., Belozersky, A., Agatow, P., Biwshich, N. and Pawlowa, M., Vergleichende Untersuchungen über Organeiweisz von Pflanzen. Hoppe-Seylers Z. Physiol. Chem. **226**, 73–86 (1934).

12 Mulder, E. G., Mineral nutrition in relation to the biochemistry and physiology of potatoes. Plant and Soil **2**, 59–121 (1949).

13 Mulder, E. G. and Bakema, K., Effect of the nitrogen, phosphorus, potassium and magnesium nutrition of potato plants on the content of free amino-acids and on the amino-acid composition of the protein of the tubers. Plant and Soil **7**, 135–166 (1956).

14 Müller-Thurgau, H., Über Zuckeranhäufung in Pflanzenteilen imfolge niederer Temperatur. Ein Beitrag zur Kenntnis des Stoffwechsels der Pflanzen. Landwirtsch. Jahrb. **11**, 751–828 (1882).

15 Müller-Thurgau, H., Beiträge zur Erklärung der Ruheperioden der Pflanzen. Landwirtsch. Jahrb. **14**, 859–863 (1885).

16 Neuberger and Sanger, F., The nitrogen in the potato. Biochem. J. **36**, 662–671 (1942).

17 Peacock, W. H. and Brunstetter, B. C., A simple chemical test for predetermining the culinary quality of potatoes as affected by the accumulation of soluble sugars. U.S. Dept. Agr. Circ. No. **158**, Pp. 4 (1931).

18 Schreven, D. A. van, Over ontijdige knolvorming bij vroege aardappels (Premature tuber formation in early poatoes). Tijdschr. Plantenz. **55**, 290–308 (1949).

19 Schreven, D. A. van, On the physiology of tuber formation in potatoes. I. Premature tuber formation. Plant and Soil **8**, 49–55 (1956).

20 Sjollema, B. and Rinkes, I. J., Die Hydrolyse des Kartoffeleiweiszes. Hoppe-Seyler's Z. Physiol. Chem. **76**, 369–384 (1912).

21 Slack, E. B., Nitrogen constituents of the potato. Nature **161**, 211–212 (1948).

22 Steward, F. C. and Street, H. E., The soluble nitrogen fraction of potato tubers; the amides. Plant Physiol. **21**, 155–193 (1946).
23 Steward, F. C. and Thompson, J. F., Proteins and protein metabolism in plants. The Proteins, Vol. II, Part A, chapter 19, p. 513–594. Academic Press, New York (1954).
24 Terman, G. L., Effect of storage temperature and size on French fry quality, shrinkage and specific gravity on Maine potatoes. Am. Potato J. **27**, 417–424 (1950).
25 Thompson, J. F. and Steward, F. C., The analysis of the alcohol-insoluble nitrogen of plants by quantitative procedures based on paper chromatography. J. Exptl. Botany **3**, 170–187 (1952).
26 Vilmorin, Andrieux, et Cie., Les plantes potagères. Pp. 489. Paris (1883).
27 Vöchting, H., Über die Keimung der Kartoffelknollen. Botan. Z. **60**, 87–114 (1902).
28 Watada, A. E. and Kunkel, R., The variation in reducing sugar content in different varieties of potatoes. Am. Potato J. **32**, 132–140 (1955).
29 Wellensiek, S. J., Ontijdige knolvorming bij vroege aardappels. Mededeel. Landbouwhoogesch., Wageningen **27**, no. 3. Pp. 24 (1924).
30 Wellensiek, S. J., Een onderzoek naar de factoren, die ontijdige knolvorming bij vroege aardappels bepalen. Thesis Wageningen (1924); Tijdschr. Plantenz. **30**, 177–226 (1924).
31 Wright, R. C., Some physiological studies in potatoes in storage. J. Agr. Research **45**, 543–555 (1932).

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ON THE PHYSIOLOGY OF TUBER FORMATION IN POTATOES

III. INFLUENCE OF GROWTH SUBSTANCES (PHYTOHORMONES) ON TUBER AND SPROUT FORMATION OF POTATOES ASEPTICALLY GROWN IN THE DARK

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INTRODUCTION

The most common growth substances hitherto found in plants are auxin a, auxin b and indolylacetic acid. A neutral growth substance was found by Larsen[21] in ether and chloroform extracts from etiolated stems of *Pisum*, *Vicia faba* and *Helianthus*. He showed that this substance is in all probability β-indolylacetaldehyde (Larsen[22 23]).

Growth substances in potatoes have been studied in different ways. In the work, of Jahnel[18 19], Lucas[24], Söding and Funke[35] the growth substance was collected in agar blocks which had been placed on potato tissue. Guthrie[11], Boysen Jensen[2], Dostal[6], Michener[26], Hemberg[15] and Pavillard[28] used ether for the extraction of growth substance from potatoes. Moewus[27] determined the content of growth substance in the sap of tubers by utilizing the stimulating effect of these substances on the roots of cress (*Nasturtium officinale*). According to him only 1 per cent of the growth substances in the tuber is present in active form (0.1 μg per ml sap).

Jahnel[18 19] using Went's[42] agar diffusing method found more auxin in healthy tubers than in virus diseased tubers. Lucas[24], Söding and Funke[35], Söding, Köhler and Funke[36] arrived at the same conclusion.

The distribution of auxin in the potato tuber has been investigated by Jarkovaja[20], Dostal[6], Michener[26] and Hemberg[12 16]. Jarkovaja[20] found most auxin in the eyes and in the cambial part of the tuber, while the auxin content was less marked in the periderm, the minimum occurring in the centre of the tuber. Dostal[6] found more auxin in the internal bast pith than in the "cortex pericycle". According to Michener[26] in non-dormant tubers the auxin concentration is high in the large apical

buds, lower in the tissue below them. and lowest in the tissue below the lateral buds. No auxin was found in the central part of the tuber. He suggested that auxin inhibits bud growth in the dormant tuber and that reduction of the auxin by the action of ethylene chlorohydrin permits growth to proceed. Hemberg [12] showed that the content of acid auxin at different stages of sprouting is greatest in the external or periderm layer and less in the internal layers. In later work Hemberg [13 14 16] noticed next to the acid auxin also a neutral growth substance in potatoes, being an aldehyd and probably indolylacetaldehyde (Hemberg [16]). The acid growth substance of the potato is in all likelihood indolylacetic acid (Hemberg [15 16]). The occurrence of neutral growth substance is concealed during the autumn by growth-inhibiting substances, which have been ascertained to a large content in extracts from peelings of dormant potatoes. About six weeks after the maturity of the potato, the greater part of these inhibiting substances have disappeared. In further experiments Hemberg [17] found that there is an acid and a neutral growth-inhibiting substance. Indeed his work makes it most probable that not auxins, but growth-inhibiting substances are of most significance for the rest period of the potato.

Söding, Köhler and Funke [36] stated already that probably two growth-inhibiting substances are present in potato tubers. One of these is probably the same as the inactive growth substance which was called antiauxin by Funke and Söding [7]. According to these investigators the active growth substance is auxin a. Antiauxin may be transformed into auxin a and the reverse reaction is also possible. The second growth-inhibiting substance was called by Meyer [25] growth-inhibiting substance H. She found that in the sap of healthy tubers approximately 30 per cent of the total amount of growth substances is present as auxin a and heteroauxin and 70 per cent as inactive growth substance, the greatest part of it being antiauxin.

In general the production of auxin in plants takes place in the apical parts of all organs which are actively elongating, such as terminal buds or growing stems, young leaves on growing petioles, inflorescences, or flowers on growing stalks and coleoptile tips (Went [43]). The transformation of the precursor into the active form can take place in darkness (Skoog [34]). Light, however, is necessary for the formation of the auxin precursor. In sprouts of tubers developing in the dark the auxins are formed from inactive precursors which are stored in a readily available form in the tuber.

Auxin transport in the shoot is strictly polar as long as the auxin is present or applied in a concentration similar to that normally occurring in the plant. When the applied concentration exceeds the normal sufficiently, the polarity disappears.

The basipetal (apex-to-base) polarity of the transport of growth substances was proven by the interesting experiments of Beyer [1], Van der Weij [40 41], Went [42], and Went and White [44]. Cholodny [3] and Pohl [29 30], however, were of opinion that auxin moves from the seed upwards into the coleoptile, but Skoog [34] showed that it is not auxin, but an auxin precursor which is transported upwardly and which is converted into auxin in the coleoptile tip.

Thus when growth substances are applied to the tubers, as in my own experiments, acropetal transport of these growth substances is not possible, unless the applied concentration exceeds the normal by a factor of 100–1000 times. Yet when potato tubers are treated with high concentrations of growth substances sprout formation is inhibited [4 5 8 9 10 11 37 38 39]. However, low concentrations exerted a decided stimulative influence on the growth of the sprouts or young tubers. It was therefore in these cases precluded that the applied growth substance was first converted into a precursor which could move from the tuber upwards into the sprouts and then converted again into growth substance in the tips. This is also supported by the experiments of M o e w u s [27]. He showed that 10 γ β-indolylacetic acid added to 1 ml sap of potato tubers is inactivated within 24 hours at 27°C. The inactivated heteroauxin may be converted again into the active form by addition of 0.5 per cent pancreatine (Merck, Darmstadt).

EXPERIMENTAL

A. *Influence of* 2,4-*dichlorophenoxyacetic acid* (2,4-*D*)

In a preliminary experiment the effect of the sodium salt of 2,4-dichlorophenoxyacetic acid was investigated on the growth in cultures with non-pregerminated tubers of the variety Duke of York *. In this experiment no special precautions were taken for keeping the solutions sterile during the growth period. Concentrations were used of 0, 0.01, 0.05 and 0.10 ppm. Each bottle contained 250 ml solution. For each concentration 12 tubers were used.

With a sterile borer a hole of 6.5 mm diameter was made three quarters of the way through the flesh of the tubers and into this hole the end of a cotton lampwick was inserted with a sterile pair of forceps. By placing the tubers on the tops of dispensing bottles filled with the culture solutions into which the lampwicks dipped, these tubers could suck up the solutions. After several weeks tubers, allowed to germinate in this way in the dark, developed young tubers on the sprouts. These tubers were counted at intervals. The mother tubers were weighed individually before starting the experiment. To compensate for the differing weights of the mother tubers the yields of young tubers and sprouts and the total yields of each series were expressed as percentages of the weights of the mother tubers.

* For the sake of brevity tubers which had not formed sprouts are called "non-pregerminated tubers", those which had formed sprouts during storage in the dark at room temperature are called "pregerminated tubers".

As is shown in Figure 1 a marked increase was found in the number and yield of young tubers the more 2,4-D was present. No influence was noted, however, on the yield of the sprouts with these small concentrations. The total yield of sprouts and tubers increased as more 2,4-D was present in the solution.

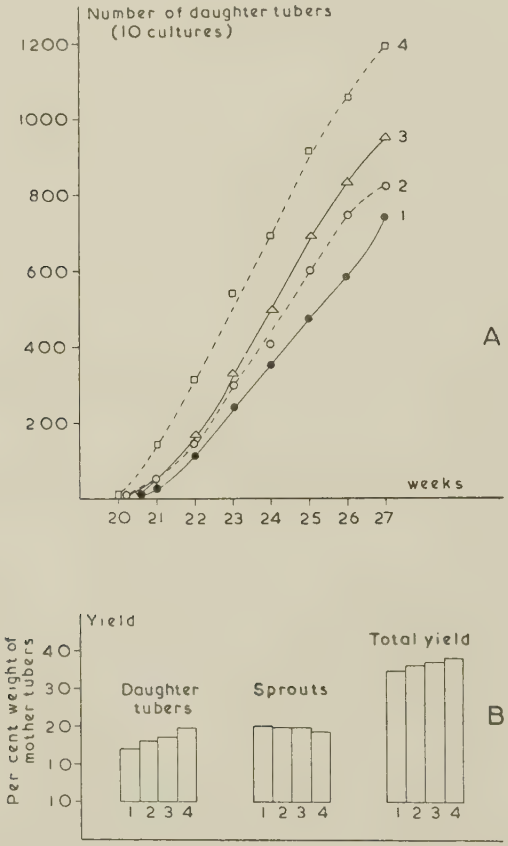

Fig. 1. *1*) Non-pregerminated tubers, grown on water (250 ml); *2*) as *1* + 0.01 ppm 2,4-D; *3*) as *2* + 0.05 ppm 2,4-D; *4*) as *1* + 0.10 ppm 2,4-D.

In Plate I a culture is shown provided with 0.1 ppm 2,4-D.

In another experiment with non-pregerminated tubers it was found that a concentration of 4 ppm 2,4-D reduced the number of daughter tubers and delayed the formation of tubers.

B. *Influence of α-naphthalene acetic acid and β-indolylacetic acid*

To investigate the influence of the uptake of these growth substances by the mother tuber under aseptic conditions the same technique was used as described in a former article [33]. The effect of the sodium salt of α-naphthaleneacetic acid (NAA) and of the sodium salt of β-indolylacetic acid (IAA) was studied in cultures

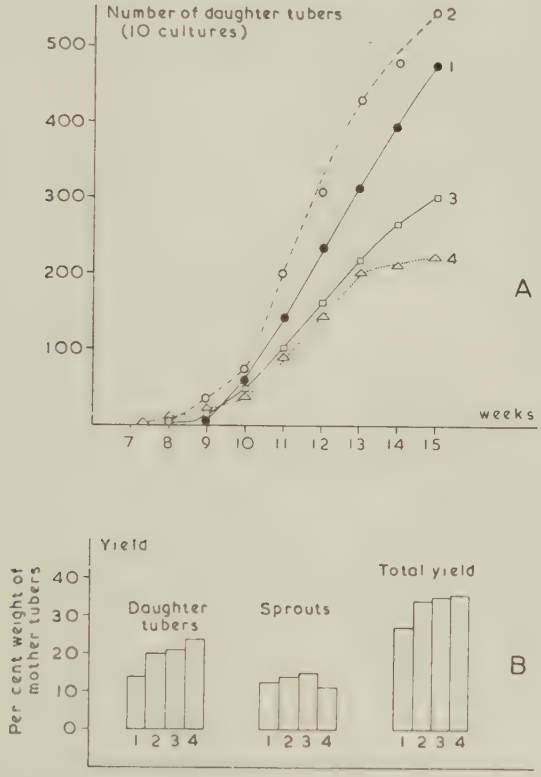

Fig. 2. *1*) Tubers kept for 8 weeks in the dark at room temperature, desprouted, grown on water (250 ml); *2*) as *1* + 0.05 ppm NAA; *3*) as *2* + 0.5 ppm NAA; *4*) as *1* + 2.0 ppm NAA.

with pregerminated and desprouted tubers of the variety Duke of York. The tubers were kept for 2 months in the dark, desprouted on 31 January 1950 and placed on bottles containing 0, 0.05, 0.5 and 2 ppm growth substance (250 ml solution).

After four weeks a decided stimulation of the growth of sprouts

was noted in the presence of the growth substances (Plate II), but at the end of the experiment the inrcease in the weight of the sprouts proved to be only very small in presence of 0.05 and 0.5 ppm NAA. As is shown in Figure 2A only the concentration of 0.05 ppm NAA caused a small increase in the number of young tubers. The numbers were decreased by the concentrations of 0.5 and 2 ppm NAA. The yield of young tubers, however, increased the more NAA was present in the solution, owing to the greater size of the daughter tubers (Plate III). The same was found with increasing amounts of IAA (Plate IV). The total yield of sprouts and tubers was increased in presence of NAA, up to 2 ppm (Figure 2B). The same effect was found with IAA (Figure 3B). No increase in the number of young tubers, however, was found in presence of 0.05 ppm IAA. As in the case with 2 ppm NAA the presence of 2 ppm IAA has advanced the formation of young tubers by some days (Figure 3A).

When the daughter tubers are kept for several months in the dark sprouts are formed. The sprout growth of daughter tubers formed on the sprouts of tubers grown on water is rather poor when the mother tubers have been pregerminated for two months and desprouted before placing them on the bottles. (Plate V*a*). When the mother tubers had not been pregerminated the sprout growth of the daughter tubers was much better (Plate V*b*). It is very interesting that the sprout growth of the daughter tubers was also much better when the mother tubers had been pregerminated and desprouted but grown on solutions containing 0.05, 0.5 or 2 ppm NAA or IAA (Plate V, *c–h*). Evidently the young tubers formed by the tubers grown on solutions containing a growth substance contained more auxin precursors or (and) growth substances than those of the control series, not supplied with growth substance. It is also evident from this experiment that the young tubers formed on the sprouts of tubers grown on water contained more auxin precursors or (and) growth substances when the mother tubers had not been pregerminated and desprouted than when this has been done. It is clear that the mother

Plate I
A non-aseptic culture of a tuber grown on a solution of 0.05 ppm of the sodium salt of 2,4-dichlorophenoxyacetic acid (2,4-D).
Plate II
Stimulated growth of sprouts of pregerminated and desprouted tubers grown for a month on solutions containing small amounts of the sodium salt of α-naphthaleneacetic acid (NAA). From left to right: solutions containing 0, 0.05, 0.5 and 2 ppm NAA.

Plate I

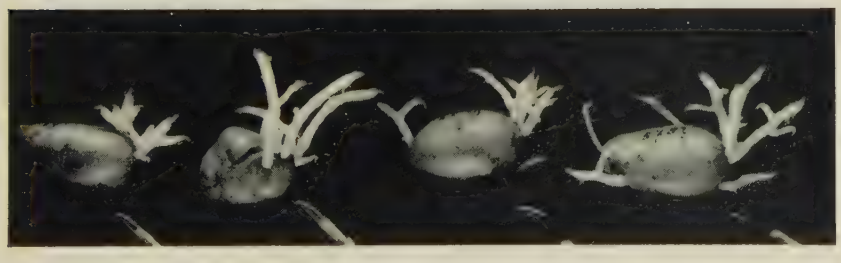

Plate II

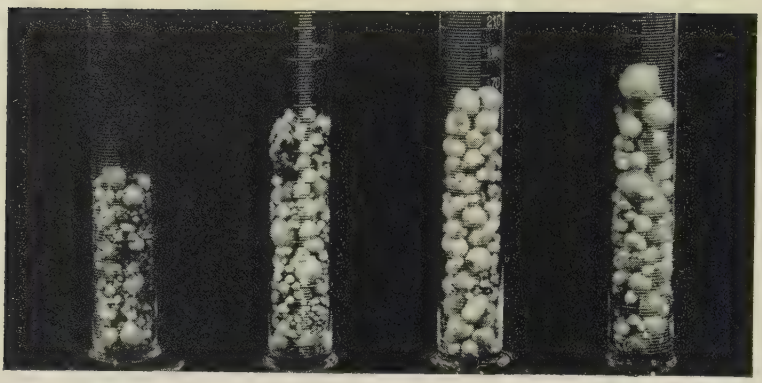

Plate III

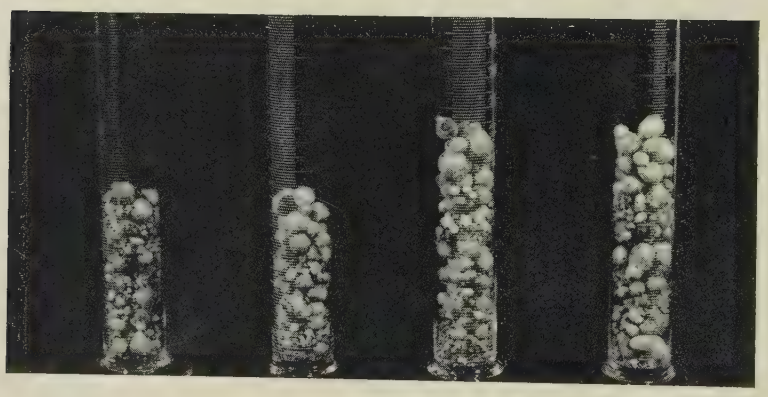

Plate IV

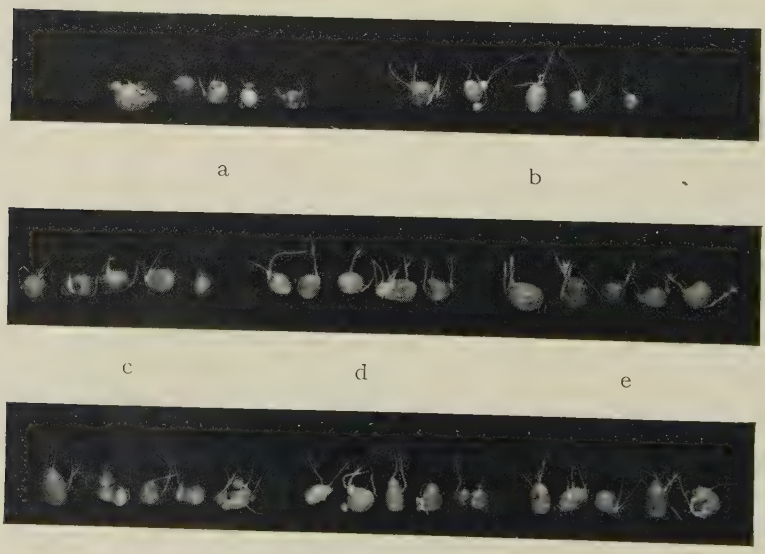

Plate V

tubers which had been pregerminated for two months in the dark at room temperature had lost an important part of the precursors and growth substances.

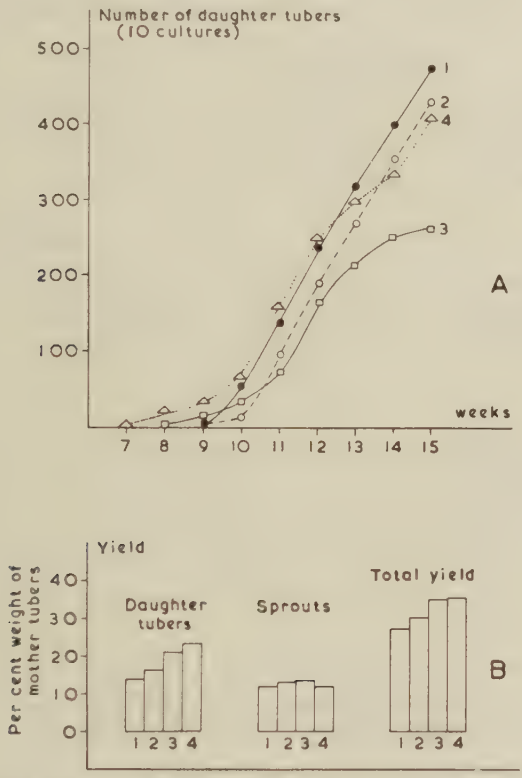

Fig. 3. *1*) Tubers kept for 8 weeks in the dark at room temperature, desprouted, grown on water (250 ml); *2*) as *1* + 0.05 ppm IAA; *3*) as *1* + 0.5 ppm IAA; *4*) as *1* + 2.0 ppm IAA.

Plate III
Effect of small amounts of the sodium salt of α-naphthaleneacetic acid (NAA) on the yield and the size of daughter tubers. The mother tubers had been pregerminated and desprouted before growing them upon the solutions. From left to right: the solutions contained 0, 0.05, 0.5 and 2 ppm NAA. Each series comprises the yield of 10 cultures.
Plate IV
The same treatment as in Plate III, but instead of solutions of NAA, solutions of β-indolylacetic acid (IAA) had been used.
Plate V
a: Daughter tubers with poor sprout-formation. These tubers had been formed in cultures with pregerminated and desprouted tubers grown on water. *b*: Daughter tubers with fair sprout-formation from cultures with non-pregerminated tubers grown on water. Middle and lower rows: daughter tubers with improved and fair sprout formation. The tubers had been formed in cultures with pregerminated and desprouted tubers grown on solutions with 0.05, 0.5 and 2 ppm NAA (*c–e*) and IAA (*f–h*).

In another experiment the influence of the sodium salt of β-indolyl acetic acid on the growth of non-pregerminated tubers was investigated. In this experiment the concentrations used were 0, 2, 4 and 8 ppm IAA (250 ml solution). As is shown in Figure 4A these con-

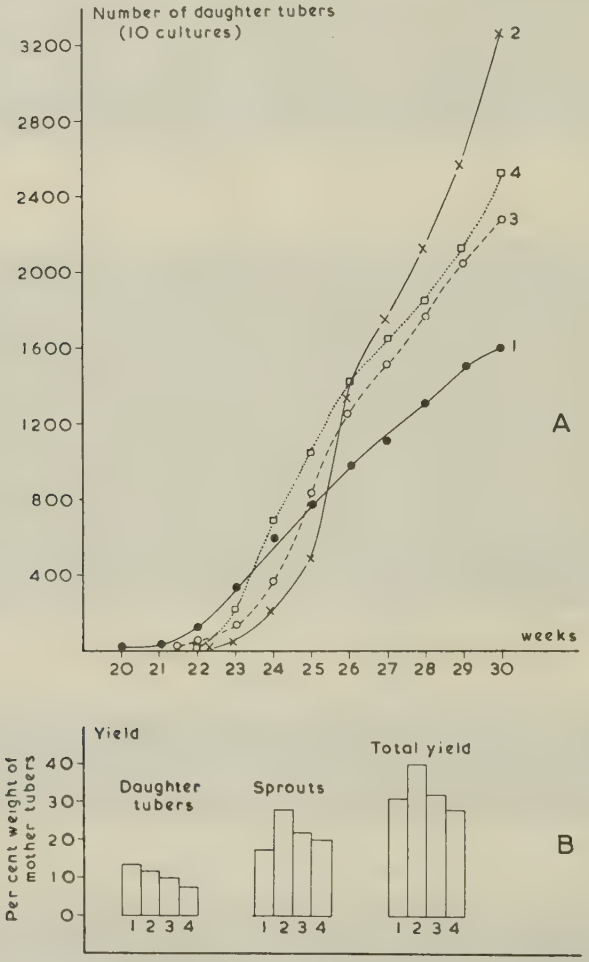

Fig. 4. *1*) Non-pregerminated tubers, grown from 8 November on water (250 ml); *2*) as *1* + 2 ppm IAA; *3*) als *1* + 4 ppm IAA; *4*) as *1* + 8 ppm IAA.

centrations caused a great increase in the number of young tubers, the number being doubled by the addition of 2 ppm IAA. The weight of the young tubers, however, as expressed in per cent weight of the

mother tubers, was decreased by this concentration. On the other hand the development of sprouts was greatly stimulated by 2 ppm IAA, so that the total yield of sprouts and tubers was much greater than in the control series. The yield of young tubers decreased gradually the more IAA was present in the solutions. The formation of young tubers was retarded for one or more weeks in presence of these amounts of IAA. It is quite possible that smaller concentrations as had been used in the experiment with 2,4-D (Figure 1) would have increased the weight of young tubers instead of the weight of sprouts.

Although the sprout formation was not depressed as compared with the control in presence of 4 and 8 ppm IAA, the growth was greatly reduced as compared with the series grown on 2 ppm IAA. The total yield of sprouts and tubers was decreased in presence of 8 ppm IAA.

C. *Influence of growth substances in combination with glucose*

The influence of growth substances in combination with glucose was investigated with tubers which had been pregerminated in the dark for 2 months from 28 November 1949 until 30 January 1950. The tubers were desprouted before placing them upon the following sterile solutions:

1. water, 250 ml (control)
2. as 1 with 0.5 ppm IAA
3. as 1 with 0.5 ppm NAA
4. as 1 with 3% glucose
5. as 1 with 3% glucose and 0.5 ppm IAA
6. as 1 with 3% glucose and 0.5 ppm NAA.

As is shown in Figure 5 the yield of young tubers was substantially increased by the growth substances and decreased in presence of

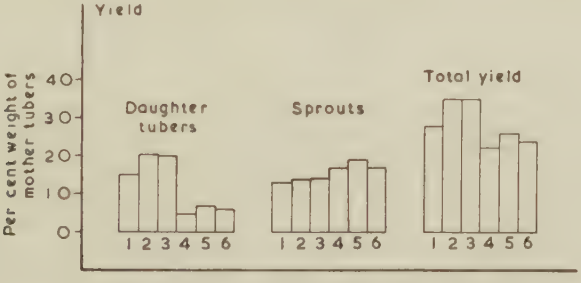

Fig. 5. *1*) Tubers kept for 8 weeks in the dark at room temperature, desprouted, grown on water (250 ml); *2*) as *1* + 0.5 ppm IAA; *3*) as *1* + 0.5 ppm NAA; *4*) as *1* + 3% glucose; *5*) as *4* + 0.5 ppm IAA; *6*) as *4* + 0.5 ppm N

glucose. The yield of the young tubers was only slightly increased by the growth substances in presence of 3% glucose. The yield of the sprouts was only very slightly or not increased by the growth substances. There was a small increase of the weight of sprouts in presence of glucose. The effect of the growth substances on sprout formation in presence of 3% glucose was only slight or absent.

The small effect of the growth substances in presence of 3% glucose is probably due to the strongly reduced uptake of solution by the tubers in presence of 3% glucose.

DISCUSSION

Tuber formation takes place earlier when sprouts have been formed during storage [31] [33]. This earlier tuber formation cannot be due to a smaller amount of auxin precursors and growth substances in the pregerminated tubers, since in the cultures with pregerminated potatoes small amounts of NAA or IAA in most cases did not delay tuber formation. On the contrary small amounts of growth substances as a rule induced earlier tuber formation in the cultures with pregerminated tubers (Figures 2 and 3). It is probable, however, that the smaller amount of precursors and growth substances in pregerminated and desprouted tubers may be responsible for a weaker development of sprouts and roots, a condition which is correlated with premature tuber formation [32].

Anyhow it is evident that in the normal growing plant growth substances play an important rôle on the development of daughter tubers.

SUMMARY

In cultures with non-pregerminated tubers a marked increase in the numbers and yield of young tubers was found in presence of small amounts of 2,4-dichlorophenoxyacetic acid (2,4-D).

In cultures with pregerminated tubers the yield of young tubers also increased considerably in presence of small amounts of the sodium salt of α-naphthaleneacetic acid (NAA) and the sodium salt of β-indolylacetic acid (IAA). Generally the formation of young tubers took place earlier in presence of small concentrations of these growth substances. In cultures with non-pregerminated tubers the number of young tubers increased considerably in presence of greater concentrations of NAA and IAA. This was attended by a smaller yield and delayed formation of young tubers.

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REFERENCES

1 Beyer, A., Beiträge zum Problem der Reizleitung. Z. Botanik **20**, 321–417 (1928).
2 Boysen Jensen, P., Quantitative Bestimmung der beschleunigenden Streckungswuchsstoffe in der sauren Fraktion der Ätherextrakte aus höheren Pflanzen. Planta **31**, 653–669 (1941).
3 Cholodny, N., Über das Keimungshormon von Gramineen. Planta **23**, 289–312 (1935).
4 Denny, F. E., Guthrie, J. D. and Thornton, N. C., Effect of the vapor of the methyl ester of α-naphthalene acetic acid on the sprouting and the sugar content of potato tubers. Contrib. Boyce Thompson, Inst **12**, 253–268 (1942).
5 Denny, F. E., The use of methyl ester of α-naphthalene acetic acid for inhibiting sprouting of potato tubers, and an estimate of the amount of chemical retained by tubers. Contrib. Boyce Thompson Inst. **12**, 387–403 (1942).
6 Dostal, R., Über das Frühtreiben der Fliederzweige (*Syringa vulgaris*) und Kartoffelknollen (*Solanum tuberosum*) durch Verletzung und die hormonale Deutung dafür. Gartenbauwiss. **16**, 195–206 (1942).
7 Funke, H. and Söding, H., Über das Wuchsstoff-Hemmstoffsystem der Haverkoleoptile und der Kartoffelknolle. Planta **36**, 341–370 (1948).
8 Guthrie, J. D., Effect of ethylene thiocyanohydrin, ethyl carbylamine and indoleacetic acid on the sprouting of potato tubers. Contrib. Boyce Thompson Inst. **9**, 265–272 (1938).
9 Guthrie, J. D., Inducing "dormancy" in potato tubers with potassium naphthaleneacetate and breaking it with ethylene chlorohydrin. Science NS. **88**, 86 (1938).
10 Guthrie, J. D., Inhibition of the growth of buds of potato tubers with the vapor of the methyl ester of naphthaleneacetic acid. Contrib. Boyce Thompson Inst. **10**, 325–328 (1939).
11 Guthrie, J. D., Control of bud growth and initiation of roots at the cut surface of potato tubers with growth regulating substances. Contrib. Boyce Thompson Inst. **11**, 29–53 (1939).
12 Hemberg, T., Änderungen des Auxingehalts in Kartoffelknollen vor und nach dem Keimen. Svensk Botan. Tidskr. **36**, 467–470 (1942).
13 Hemberg, T., Über das Vorkommen wachstumhemmender Stoffe in Kartoffelknollen und die Bildung wachstumförderender Stoffe in Wundflächen derselben. Arch. f. Botan. Stockholm **30** B, no. 7 (1943).
14 Hemberg, T., Weitere Untersuchungen über wachstumförderende Stoffe in Wundflächen von Kartoffelknollen. Svensk Botan. Tidskr. **38**, 428–430 (1944).
15 Hemberg, T., Wachstumshemmende und wachstumförderende Stoffe bei der Kartoffel. Arch. f. Botan. Stockholm **33** B, no. 2, pp. 3 (1946).
16 Hemberg, T., Studies of auxins and growth-inhibiting substances in the potato tuber and their significance with regard to its rest-period. Acta Horti Bergiani **14**, 133–220 (1947).
17 Hemberg, T., Significance of growth-inhibiting substances and auxins for the rest-period of the potato tuber. Physiol. Plantarum **2**, 24–36 (1949).
18 Jahnel, H., Wuchstoffuntersuchungen an abbaukranken Kartoffeln. I. Phytopathol. Z. **10**, 113–117 (1937).
19 Jahnel, H., Wuchstoffuntersuchungen an abbaukranken Kartoffeln. II. Phytopathol. Z. **12**, 312–317 (1939).
20 Jarkovaja, L. M., Changes in the content of growth substances due to interruption of the period of rest. Compt. Rend. (Doklady) Acad. Sci. U.S.S.R. **23**, 88–91 (1939).
21 Larsen, P., Skototenin, ein neuer Streckungswuchsstoff in höheren Pflanzen. Naturwiss. **27**, 549–550 (1939).

22 Larsen, P., Beta-indolyl-acetaldehyd als Streckungswuchsstoff in höheren Pflanzen. Botan. Tidsskr. **46**, 146–147 (1943).
23 Larsen, P., 3-Indole acetaldehyde as a growth hormone in higher plants. Dansk Botan. Arch. **11**, 11–132 (1944).
24 Lucas, H., Weitere Untersuchungen über den Wuchstoffhaushalt abbaukranker Kartoffeln. Phytopathol. Z. **12**, 334–350 (1940).
25 Meyer, E., Über den Wuchsstoff-Hemmstoffgehalt gesunder und abbaukranker Kartoffelknollen. Planta **38**, 213–232 (1950).
26 Michener, H. D., Dormancy and apical dominance in potato tubers. Am. J. Botany **29**, 558–568 (1942).
27 Moewus, F., Gebundener und freier Wuchstoff in der Kartoffelknolle. Z. Naturforschung **3** B, 135–136 (1948).
28 Pavillard, J., L'extraction à l'éther, methode de dosage des substances de croissance chez la pomme de terre. Compt. Rendu de l'Acad. des Sciences **224**, 1840–142 (1947).
29 Pohl, R., Über den Endospermwuchsstoff und die Wuchsstoffproduktion der Koleoptilspitze. Planta **24**, 523–526 (1935).
30 Pohl, R., Die Abhängigkeit des Wachstums der Avena-Koleoptile und ihrer sogenannten Wuchsstoffproduktion von Auxingehalt des Endosperms. Planta **25**, 720–750 (1936).
31 Schreven, D. A. van, Over ontijdige knolvorming bij vroege aardappels. (Premature tuber formation in early potatoes). Tijdschr. Plantenz. **55**, 290–308 (1949).
32 Schreven, D. A. van, On the physiology of tuber formation in potatoes. I. Premature tuber formation. Plant and Soil **8**, 49–55 (1956).
33 Schreven, D. A. van, On the physiology of tuber formation in potatoes. II. Influence of some organic compounds on tuber and sprout formation of potatoes aseptically grown in the dark. Plant and Soil **8**, 56–74 (1956).
34 Skoog, F., A deseeded Avena test method for small amounts of auxin and auxin precursors. J. Gen. Physiol. **20**, 311–334 (1937).
35 Söding, H. and Funke, H., Über den Wuchsstoffhaushalt abbaukranker Kartoffeln. Phytopathol. Z. **13**, 351–368 (1941).
36 Söding, H., Köhler, E. and Funke, H., Über den Wuchsstoffgehalt abbaukranker Kartoffeln. Phytopathol. Z. **14**, 427–441 (1943).
37 Stuivenberg, J. H. M. and Veldstra, H., De practische toepassing van remming der spruitvorming bij aardappelen door behandeling met groeistoffen in dampvorm. Landbouwk. Tijdschr. **54**, 611–632 (1942).
38 Thomas, J. E. and Riker, A. J., Sprouting of potatoes inhibited by plant hormones. Am. Potato J. **27**, 417–424 (1950).
39 Veldstra, H., Onderzoekingen over plantengroeistoffen. I. Practische toepassingen. Remming van de spruitvorming bij aardappelen door behandeling met groeistoffen in dampvorm. Landbouwk. Tijdschr. **54**, 10–22 (1942).
40 Weij, H. G. van der, Der Mechanismus des Wuchsstoffstransportes. Rec. Trav. Botan. Néerl. **29**, 379–496 (1932).
41 Weij, H. G. van der, Der Mechanismus des Wuchsstofftransportes II. Rec. Trav. Botan. Néerl. **31**, 810–857 (1934).
42 Went, F. W., Wuchsstoff und Wachstum. Rec. Trav. Botan. Néerl. **25**, 1–116 (1928).
43 Went, F. W. and Thimann, K. V., Phytohormones. The Macmillan Comp., New York, 1–294 (1937).
44 Went, F. W. and White, R., Experiments on the transport of auxin. Botan. Gaz. **100**, 465–484 (1939).